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The use of sampling weights in regression models of recreational fishing-site choices

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Abstract—Improved methods for estimating saltwater recreational fishing catch and effort have been developed by the NOAA National Marine Fisheries Service. Sampling weights that account for a complex sample design in surveys of anglers are now available with NMFS catch and effort estimates. Previously, estimates of the economic value to anglers (known as the "willingness to pay") for additional fish caught that were based on angler surveys did not typically account for the underlying complex sample design. In this study, a recreational-demand model was used for analysis of fishing site choices in the Gulf of Mexico in 2009 among private-boat anglers who target groupers (*Epinephelus* spp., *Hyporthodus* spp., or *Mycteroperca* spp.) or red snapper (*Lutjanus campechanus*). Different versions of the model were developed with and without accounting for the complex sample design. Results between the unweighted version and weighted versions of the model varied in estimates of catch between sites and the value anglers place on being able to catch and keep additional fish.

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In 2012, the NOAA National Marine Fisheries Service (NMFS) released a new method for estimation of recreational fishing catch and effort based on data obtained from its Access Point Angler Intercept Survey (APAIS) of saltwater anglers. Previous methods of estimation of catch and fishing effort from this intercept [interview] survey were subject to a number of different potential biases as pointed out by the National Research Council of the National Academies (NRC, 2006). In particular, the earlier estimation methods did not account for the complex sample design of the intercept survey and instead simple random sampling was assumed. The new method of estimating catch and effort uses specially calculated weights and variance adjustments (Breidt et al.[1]).

The APAIS sampling weights incorporate information from a separate survey, the NMFS Coastal Household Telephone Survey (CHTS), that is used to estimate fishing effort by coastal residents by state, wave (defined as a consecutive 2-month period), and fishing mode (private boat and shore). Data from the APAIS on the proportion of angler effort from coastal residents to angler effort from noncoastal and out-of-state residents are used to scale the level of angler effort from coastal residents up to an unbiased estimate of total effort for all anglers, both coastal and noncoastal. For example, 85% of private boat trips that targeted groupers and red snapper in the Gulf of Mexico in 2009 were taken by residents of coastal counties. Inclusion of the APAIS sampling weights in recreational site-choice demand models will ensure that results correctly reflect the true proportion of trips that come from coastal residents compared with trips from noncoastal residents. This inclusion is important because the costs associated with traveling between an angler's home and different fishing sites used in the demand models will vary on the basis of proximity to the coast.

A number of recreational site-choice demand models have been developed with the APAIS data (e.g., Whitehead and Haab, 2000; Gentner, 2007; Haab et al., 2012). These mod-

[1] Breidt, F. J., H.-L. Lai., J. D. Opsomer, and D. A. Van Voorhees. 2012. A report of the MRIP sampling and estimation project: improved estimation methods for the Access Point Angler Intercept Survey component of the Marine Recreational Fishery Statistics Survey. [Available from Fisheries Statistics Division, Natl. Mar. Fish. Serv., NOAA, Silver Spring, MD, and from http://www.countmyfish.noaa.gov/projects/downloads/Final%20Report%20of%20New%20Estimation_Method_for_MRFSS_Data-01242012.pdf.]

els examine how anglers make choices about which fishing sites to visit on the basis of the costs of travel to a site and the qualities of a site. For models of recreational fishing, site quality is typically measured by the average harvest rate per angler at a site. The parameters of site-choice models are used to estimate economic values associated with recreational fishing. However, failure to account for the complex sampling design of the NMFS APAIS survey could result in biased demand-model parameters. It is important to have unbiased model parameters to obtain accurate estimates of benefits and costs and to ensure that policy recommendations are not misleading.

The APAIS data are collected by using a stratified, multistage survey design with stratification that is based on intercept site, time of year, and other variables. Therefore, the proportion of anglers interviewed at each intercept site may reflect sampling allocations and not necessarily reflect angler preferences or the *demand* for one site over another. This problem of demand estimation is commonly referred to as *endogenous stratification*. Another issue with demand estimation can arise when using APAIS data because more avid users tend to be overrepresented in intercept surveys. This problem, referred to as *avidity bias*, can cause demand-model parameters to be influenced more heavily by avid users. Hindsley et al. (2011), using simulated data sets, found evidence of both endogenous stratification and avidity bias. However, their analysis was performed before the new NMFS estimation methods were available. The sampling information made available through the updated estimation methods can be used to generate sampling weights to correct for endogenous stratification in recreational site-choice demand models developed with data from the APAIS.

The goal of our analysis was to compare the estimates of parameters and economic value that result from the use of a typical NMFS recreational site-choice demand model with and without the newly available sampling weights designed to correct for endogenous stratification. We used a model of fishing site choices among private-boat anglers in the Gulf of Mexico who target groupers (*Epinephelus* spp., *Hyporthodus* spp., or *Mycteroperca* spp.) or red snapper (*Lutjanus campechanus*). Following Kuriyama et al. (2013), we focus on the correction for endogenous stratification and do not attempt to correct for potential avidity bias. More details on avidity bias and how to correct for it are given in Thomson (1991) and Hindsley et al. (2011).

Materials and methods

Model specification and estimation of angler willingness to pay

The standard recreational discrete choice model that uses APAIS data has the angler choosing a preferred fishing site on any given occasion when a choice can be made (e.g., day). Following Whitehead and Habb (2000) and Gentner (2007), we limited the options available to each angler to trips at locations within a 300-mi (483 km) round trip from an angler's residence. We also assumed that the angler had already decided to fish from a private boat and had decided which species to target so that the primary choice was where to launch the boat. In our model, this choice is made by comparing the benefits or *utility* available from each potential launch site against the costs of getting to each site. The indirect utility, U_j, of going to site j for angler i can be written as

$$U_{ji} = v_{ji}\,(q_j,\, m_i - c_{ji}) + \varepsilon_{ji}, \quad (1)$$

where m_i = income; and

for angler i at site j:

v_{ij} = the observable portion of utility;
c_{ij} = the trip cost;
q_j = a vector of attributes that defines the quality of fishing and other site features; and
ε_{ij} = the error term that represents the unobserved (to the analyst) portion of utility.

The observable portion of utility, such as travel costs and site characteristics (harvest rates for a site) or other site amenities (such as those at a marina), is based on those attributes that can be observed and measured by the analyst. The unobserved portion includes information on characteristics of the site or angler that are unavailable to the analyst, for example, the presence of a tackle shop near a site or the number of years of experience an angler has at a given site.

Under the assumptions of the random utility model (McFadden, 1974), an angler will choose the site that provides the greatest level of utility:

$$V_{ij}\,(q_j,\, m_i - c_{ij}) + \varepsilon_{ij} \geq V_{is}(q_s,\, m_i - c_{is}) + \varepsilon_{is} \;\; \forall\, j \neq s, \quad (2)$$

where V = the utility function; and
j = a member of s recreation sites.

Assuming that the observed portion of utility is linear, $V_{ij}\,(q_j,\, m_i - c_{ij}) = \beta_q q_j + \beta_c c_{ij}$, and the unobserved portions of utility, ε_j, have a type-I extreme value distribution, the probability that angler i chooses site j can be estimated with a standard conditional logit model:

$$P_{ij} = P(y_i = j) = \frac{\exp(\beta_q q_j + \beta_c c_{ij})}{\sum_{s=1}^{J} \exp(\beta_q q_j + \beta_c c_{ij})}, \quad (3)$$

where y_i = the choice made by angler I; and
β_q and β_c are parameters to be estimated.

The parameters of the conditional logit model are typically estimated through the use of maximum likelihood with the following log-likelihood expression:

$$LL(\beta) = \sum_{i=1}^{N} \sum_{j=1}^{J} d_{ij} \log P_{ij}, \quad (4)$$

where N = the number of anglers in a sample; and

d_{ij} = 1 if angler i chooses site j and 0 otherwise.

We will refer to Equation 4 as the *unweighted estimator* because this specification is based on the assumption that the anglers are selected to be interviewed by simple random sampling. Manski and Lerman (1977) suggested a way to weight the conditional logit estimator by using information on the estimated population proportion of anglers observed at each of the j sites. The log likelihood of the weighted exogenous sampling maximum likelihood (WESML) estimator is

$$LL(\beta) = \sum_{i=1}^{N} \sum_{j=1}^{J} \frac{Q_j}{H_j} d_{ij} \log P_{ij}, \quad (5)$$

where Q_j and H_j are the population and sample proportions at site j.

Note that the variance matrix of the estimated parameters must also be corrected to reflect the nonrandom nature of the data (Lerman and Manski, 1981).

The estimated parameters of the conditional logit model can be used to determine an angler's willingness to pay (WTP) for changes in site characteristics or for the loss or addition of a site (Haab and McConnell, 2002). The most common measure used in the NMFS recreational site-choice demand models with the APAIS data is the value of a one-unit change in harvest rate. If we define one element of q, say q_1, as the harvest rate, then the average angler WTP for a one-unit change in this attribute is given by

$$WTP(q_1) = \frac{\beta_{q_1}}{\beta_c}. \quad (6)$$

We calculated confidence intervals for this nonlinear combination of parameters using the method of Krinsky and Robb (1986) with 1000 random draws from the multivariate normal distribution defined by the estimated mean parameter vector β and its related estimated covariance matrix. The mean marginal WTP by anglers is given by the mean of the simulated WTP vector calculated by evaluating expression 5 with the 1000 parameter draws. Similarly, the upper and lower 95% confidence intervals are given by the 5th and 95th quantile of the simulated WTP vector. The unweighted conditional logit and the WESML versions of the model and WTP confidence intervals were estimated with the software NLogit[2], vers. 5 (Econometric Software, Plainview, NY).

The simulated WTP vectors can also be used to formally test whether the mean WTP estimates generated by the weighted versions of the model are significantly different from the mean WTP estimates produced with the unweighted version of the model. We use the method of convolutions suggested by Poe et al. (2005) to test the null hypothesis that the estimates of mean unweighted WTP and mean weighted WTP are equal against the alternative that the unweighted estimate is larger than the weighted estimate. The calculations with the method of convolutions were performed with the mded package for R, vers. 3.0.0 (R Core Team, 2013).

Sources and description of data

Data with which to estimate the conditional logit demand models came from a sample of private-boat anglers who were interviewed in 2009 as part of the APAIS in the Gulf of Mexico. The APAIS is a stratified, multistage, cluster-sample survey of angler trips designed to obtain estimates of mean catch per angler fishing day by species type and angler effort for each state, wave (2-month period), fishing mode (private boat, for-hire boat, and shore), and area fished (inland, state waters, and federal waters). The sample frame is based on the number of days at coastal fishing sites (site-days) that are accessible to the general public. Before 2011, simple random sampling was assumed during estimation procedures for estimates of mean catch and total effort, although the data were collected through the use of a complex sample design. The APAIS sampling weights were designed to incorporate the complex sample design and nonrandom nature of the sample (Breidt et al.[1]). Available as part of the APAIS data sets, these weights were calibrated by the NMFS Marine Recreational Information Program so that they summed to total estimated angler effort by year, wave, state subregion, fishing mode, and area fished. This calibration was based on estimates of total angler effort from data from the APAIS and CHTS, both of which are part of the NMFS Marine Recreational Information Program. The CHTS is a random-digit-dial telephone survey of households stratified by coastal counties to obtain the number of private boat and shore trips made by each member of a household in a 2-month period. Summation of the weightings within or across strata can then provide an estimate of total fishing effort by those same strata or combinations of strata (Foster[3]).

We selected APAIS trips by anglers who launched from sites along the coasts of western Florida, Alabama, Mississippi, and Louisiana and who reported targeting (by hook and line) red snapper or any of the following species of groupers: rock hind (*Epinephelus adscensionis*), speckled hind (*E. drummondhayi*), red hind (*E. guttatus*), red grouper (*E. morio*), yellowedge grouper (*Hyporthodus. flavolimbatus*), misty grouper (*H. mystacinus*), Warsaw grouper (*H. nigritus*), snowy grouper (*H. niveatus*), black grouper (*Mycteroperca bonaci*), yellowmouth grouper (*M. interstitialis*), gag (*M. microlepis*), scamp (*M. phenax*), tiger grouper (*M. tigris*), and yellowfin grouper (*M. venenosa*). Anglers who indicated they targeted any type of grouper also were

[2] Mention of trade names or commercial companies is for identification purposes only and does not imply endorsement by the National Marine Fisheries Service, NOAA.

[3] Foster, J. 2013. Personal commun. Office of Science and Technology, Natl. Mar. Fish. Serv., NOAA, Silver Spring, MD 20910.

Table 1

Average unweighted (UW) and weighted (W) harvest rates per angler trip for groupers (*Epinephelus* spp., *Hyporthodus* spp., or *Mycteroperca* spp.) and red snapper (*Lutjanus campechanus*) were calculated for 28 fishing zones, which represented a coastal county along the Gulf of Mexico where fishing for these species occurred in 2009. Data are from the National Marine Fisheries Service's Access Point Angler Intercept Survey in west Florida, Alabama, Mississippi, and Louisiana for anglers fishing from private boats. This table also shows the number of individual sites within each zone where fishing occurred for groupers and red snapper. The proportion of angler trips by zone is shown for the sample (H_j) data. Survey weights were used first to estimate the total number of trips at the population level by zone and then to calculate by zone the proportion of trips evaluated at the mean population level (Q_j) and the lower and upper limits of the 95% confidence interval around the mean. No value in a cell signifies that the zone had no predicted trips in the lower-limit scenario and, therefore, data for that zone were not used.

		Harvest rate				Proportion of trips			
		Groupers		Red snapper			Population (Q_j)		
Zone	Number of sites	UW	W	UW	W	Sample (H_j)	Mean	Lower limit	Upper limit
1	7	0.150	0.043	1.155	1.003	0.025	0.020	0.036	0.016
2	3	0.708	0.763	1.000	0.898	0.046	0.038	0.046	0.035
3	9	0.732	0.668	1.273	1.219	0.023	0.050	–	0.068
4	2	0.532	0.527	0.010	0.010	0.002	0.004	–	0.006
5	5	0.433	0.411	0.010	0.010	0.058	0.040	0.048	0.037
6	7	0.059	0.049	0.010	0.010	0.033	0.054	0.047	0.053
7	4	0.563	0.533	0.333	0.333	0.003	0.003	–	0.005
8	11	0.403	0.408	1.380	1.601	0.074	0.072	0.059	0.071
9	11	1.119	1.098	1.310	1.495	0.036	0.059	–	0.071
10	3	0.864	0.956	1.086	1.181	0.007	0.003	–	0.005
11	3	0.246	0.222	0.010	0.010	0.085	0.053	0.060	0.049
12	9	0.148	0.262	0.010	0.010	0.104	0.091	0.137	0.078
13	7	0.254	0.165	0.010	0.010	0.006	0.007	–	0.010
14	3	1.027	1.435	0.010	0.010	0.003	0.002	–	0.003
15	7	0.193	0.212	0.010	0.010	0.034	0.034	0.011	0.037
16	5	0.208	0.303	1.153	1.188	0.030	0.039	0.022	0.041
17	4	0.384	0.318	0.010	0.010	0.042	0.033	0.045	0.029
18	19	0.344	0.337	1.500	1.643	0.187	0.188	0.351	0.148
19	3	0.182	0.193	1.262	1.689	0.026	0.056	–	0.084
20	6	0.551	0.499	1.402	1.402	0.067	0.058	0.044	0.058
21	5	1.143	1.054	0.010	0.010	0.002	0.001	–	0.002
22	5	0.786	0.749	0.010	0.010	0.031	0.043	0.041	0.042
23	3	0.010	0.010	1.020	1.045	0.022	0.018	0.021	0.017
24	3	0.010	0.010	1.707	1.381	0.002	0.001	–	0.001
25	3	1.400	1.039	1.749	1.867	0.018	0.018	–	0.023
26	2	0.010	0.010	2.931	3.412	0.008	0.006	–	0.007
27	4	0.010	0.010	0.200	0.170	0.006	0.002	0.006	0.001
28	5	0.010	0.010	0.512	0.520	0.017	0.009	0.026	0.005

included. Anglers from Texas were not included because Texas does not participate in the Marine Recreational Information Program. Following standard practice (e.g., Gentner, 2007; Haab et al., 2012), we grouped together the intercept sites, where anglers were interviewed, within the same coastal county and then redefined the site chosen by the angler in terms of a coastal county, or *zone*. Across the 4 states in our sample, 160 APAIS intercept sites had data for private-boat trips for groupers or red snapper. We aggregated these 160 sites into 28 zones (Table 1). To address potential biases with aggregation of differing numbers of sites in each zone, a variable for the natural logarithm of the number of sites in that zone was included in the vector, q, of attributes (Parsons and Needleman, 1992). Figures 1 and 2 were created with the maps, ggplot2, and GISTools packages for R, vers. 3.0.0 (R Core Team, 2013).

The mean population-level frequency of trips in each zone in 2009 (and the 95% confidence interval) was estimated with the Surveyfreq procedure in SAS, vers. 9.3 (SAS Institute, Cary, NC) by using the APAIS weightings, stratum, and cluster information. The mean, lower confidence limit, and upper confidence limit of the population proportions (Q_j) were divided

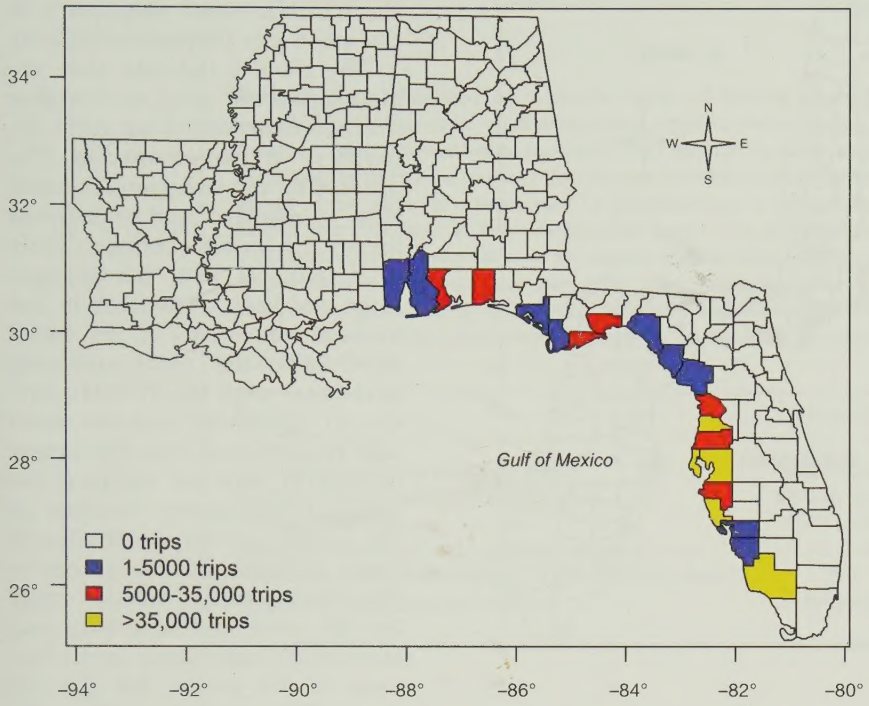

Figure 1

The number of angler fishing trips, by state, that targeted groupers (*Epinephelus* spp., *Hyporthodus* spp., or *Mycteroperca* spp.) from private boats in the Gulf of Mexico in 2009. Data are from the National Marine Fisheries Service's Access Point Angler Intercept Survey. Trips are distributed across coastal counties in Florida, Alabama, Mississippi, and Louisiana according to locations of intercept sites on shore where anglers were interviewed for the survey.

by the sample proportions (H_j) to construct 3 versions of the weights for the WESML estimator (Table 1). On the basis of the lower limit of the population proportion, 12 of the 28 zones had no predicted trips in the scenario; these zones were left out of the model in the lower-limit scenario. In other words, whereas the other models were estimated with 28 zones, the lower-limit WESML model was estimated with 16 zones.

We used APAIS catch data to calculate the unweighted and weighted average harvest rates for groupers and red snapper for each of the 28 zones over the 5 years (2004–08) that preceded our sample period of 2009 (Table 1). Average harvest rates were calculated with the Surveymeans procedure in SAS. These targeted harvest rates represent the number of fish caught and kept per angler on a targeted trip and are used as a proxy for fishing quality in the vector, q, of attributes in the demand model. We also considered including the catch rates for other snappers (as a group) into the model as did Haab et al. (2012). However, there were very few target trips for offshore varieties of snappers in the APAIS data set (gray snapper [*Lutjanus griseus*] was excluded because it is primarily an inshore species). Zones with historically high targeted harvest rates for groupers and red snapper should be preferred by anglers targeting these species. Note that the unweighted conditional logit demand model was based on the unweighted harvest rates, whereas the WESML demand models were based on the weighted harvest rates. Our goal was to compare estimated parameters and angler WTP between a typical demand model that does not use any APAIS weight information and typical demand models that do use this information.

Travel cost, c, to each zone was calculated as the round-trip distance in miles, from the centroid of the angler's zip code of residence to the centroid of the first zip code in the zone, multiplied by the cost per mile. The distances were calculated with PC*Miler, vers. 23 (ALK Technologies, Princeton, NJ). We used $0.59 as the cost per mile on the basis of the standard business mileage rate for 2009 from the Internal Revenue Service (IRS[4]), adjusted to 2012 dollars using the Consumer Price Index (CPI Inflation Calculator, Bureau of Labor Statistics, http://www.bls.gov/data/inflation_calculator.htm). We did not include the opportunity cost of time in the model because information on angler income or whether or not the angler took time off from work to fish was not collected in the 2009 APAIS. Gentner (2007) notes that relatively few (e.g., <4% in 2000) anglers reported having foregone income when asked to be part of the APAIS.

Results

In 2009, anglers who targeted primarily groupers took trips from central and southwestern Florida (Fig. 1). Anglers in Louisiana and Mississippi targeted red snapper more often than groupers (Fig. 2). In the Florida panhandle area and in Alabama, anglers tended to target both species, but the proportion of trips during which groupers were targeted there was less than the proportion of trips in other areas of Florida. In the models, 990 observations were available for use; 725 trips during which anglers targeted groupers and 265

[4] IRS (Internal Revenue Service). 2008. IRS announces 2009 standard mileage rates. IRS News Release IR-2008-131, 24 November. [Available from http://www.irs.gov/uac/IRS-Announces-2009-Standard-Mileage-Rates.]

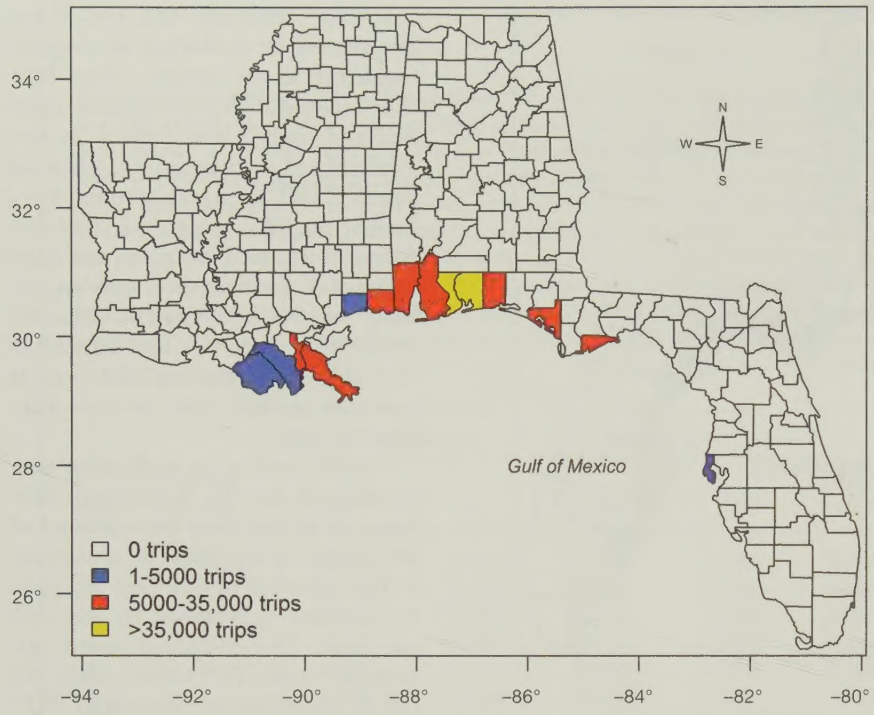

Figure 2

The number of angler fishing trips, by state, that targeted red snapper (*Lutjanus campechanus*) from private boats in the Gulf of Mexico in 2009. Data are from the National Marine Fisheries Service's Access Point Angler Intercept Survey. Trips are distributed across coastal counties in Florida, Alabama, Mississippi, and Louisiana according to locations of intercept sites on shore where anglers were interviewed for the survey.

trips during which anglers targeted red snapper (Table 2). With application of the sampling weights, these observations correspond to an estimated 716,701 trips. Anglers that targeted groupers traveled an average of 53 mi (85 km) round trip between their permanent residence and the fishing site, whereas those anglers that targeted red snapper averaged 72 mi (116 km) round trip. Anglers caught an average of 0.39 groupers per trip in the previous 5 years and an average of 1.38 red snapper per trip.

The estimation results of the site-choice model show that regardless of model version, anglers preferred sites that had lower travel costs than other sites in their choice set (Table 3). The parameter (β_c) related to the travel cost variable was statistically significant and similar in magnitude across all versions of the model. In the unweighted version of the model, the estimated travel cost parameter was −0.060, versus −0.056 for the version of the WESML model weighted with the mean site frequencies (Table 3). The results also indicate that anglers were relatively more likely to be observed fishing from zones with a relatively higher number of fishing sites. The size of the parameter for the site aggregation variable was fairly consistent between the unweighted version of the model (0.251) and the WESML model weighted with the mean site frequencies (0.242).

The results indicate that anglers preferred sites with higher average catch-and-keep rates regardless of target species. The parameter for the grouper catch rate is positive and significant across all model versions (Table 3). Compared with the grouper catch-and-keep parameter in the unweighted version of the model (b_1=0.784), the catch-and-keep parameter with the WESML version of the model with the mean site frequencies was 4% larger (b_1=0.819). For red snapper, the comparisons across versions of the model had results similar to those comparisons for groupers. The estimated parameter value for the catch-and-keep rate was statistically significant in all versions of the model. For the red snapper catch-and-keep rate, the unweighted version of the model resulted in a parameter estimate of 0.471. In contrast, as with the grouper catch-and-keep rate, the parameter estimate for the red snapper catch-and-keep rate from the WESML version of the model weighted with the mean site frequencies was higher (b_1=0.603).

For the travel cost variable, in comparison with the unweighted version of the model, weighting with the lower-limit site frequencies resulted in lower estimated parameter values whereas the use of the upper-limit site frequencies resulted in higher estimated parameter values. The same pattern was observed with the parameters of the harvest rate variables. Ultimately, however, we were interested in the net effect that sampling weights had on the estimates of mean marginal WTP for changes in harvest rates.

The mean marginal WTP for groupers varied between $8 and $15 and was significant in all model versions (Table 4). The mean WTP for an additional grouper estimated with the model version that used the weighted catch rates plus the correction for choice-based sampling at the mean frequencies was $14.67, a value 12.8% higher than the estimate of $13.01 from the unweighted version of the model. For red snapper, the mean marginal WTP varied between $4.50 and $13.81 across the different versions. For this species, the model that used weighted catch rates plus the correction for choice-based sampling at the mean frequencies had a WTP estimate of $10.81. This value was 38.6% higher than the estimate of $7.81 from the unweighted version of the model. These results indi-

Table 2

Factors that influenced an angler's choice of fishing site for groupers (*Epinephelus* spp., *Hyporthodus* spp., or *Mycteroperca* spp.) or red snapper (*Lutjanus campechanus*) targeted from private boats in the Gulf of Mexico in 2009: average round-trip distance (in miles) between an angler's permanent residence and a chosen fishing site (standard errors of the mean are presented in parentheses), average travel cost, harvest rate per angler trip (number of fish caught and kept), and average number of fishing sites in a zone (coastal county) where an angler fished. Also shown are the number of angler interviews in which anglers indicated they had targeted groupers or red snapper on the intercepted fishing trip. Data are from the National Marine Fisheries Service's Angler Access Point Intercept Survey in Louisiana, Mississippi Alabama, and west Florida in 2009.

Factor	Groupers	Red snapper
Average round-trip distance (mi)	52.61 (5.19)	72.32 (10.84)
Average travel cost (in 2009 dollars)	$26.30 (2.60)	$36.16 (5.42)
Average harvest rate	0.39 (0.03)	1.38 (0.07)
Average number of fishing sites per zone	9.86 (0.61)	6.42 (0.80)
Number of interviews (trips) in 2009 APAIS data	725	265
Estimated total number of trips	499,931	216,770

cate that weighting may matter with regard to the parameter and WTP estimates. However, on the basis of results from the method of convolutions, we cannot reject the null hypothesis that the unweighted and weighted estimates are equal at the 5% significance level. The *P*-values from testing the unweighted versus the weighted WTP estimates for groupers at the mean, lower-limit, and upper-limit estimated population weights were 0.66, 0.16, and 0.71, respectively. Similarly, the *P*-values from testing the unweighted versus the weighted WTP estimates for red snapper at the mean, lower-limit, and upper-limit estimated population weights were 0.91, 0.09, and 0.99, respectively.

Discussion

The primary aim of this research was to examine estimates of angler WTP that are derived from recreational demand models based on the APAIS data with information from the new sampling weights. We used weighted and unweighted data to estimate different versions of a model of recreational site-choice and measures of angler WTP for changes in harvest rates for groupers and red snapper in the Gulf of Mexico. The structure of the models was selected so that it would be as consistent as possible with the standard site-choice models used for policy-making at NMFS.

Depending on the weights used and species considered, the difference in estimates of angler WTP for a one unit increase in the harvest rate between the weighted versions of the site-choice model and the unweighted version of the model ranged from 13% to 77%. However, by conventional standards, none of the weighted estimates were statistically different from the corresponding unweighted estimates. Although not statically different, the variation could appear large when examining estimates across all anglers that targeted these species. Interpretation of aggregate estimates should be done with care so as not to misdirect policy recommendations. Using APAIS data for the southeastern United States in 2003–04 but different types of weights, Hindsley et al. (2011) found that weighted estimates of angler WTP were around 40% lower than unweighted estimates. Yet, they did not formally test the differences. Kuriyama et al. (2013) found that weighted estimates were around 30% lower than unweighted estimates of angler WTP for changes in harvest rates by shore anglers in California. The data used by Kuriyama et al. (2013) were similar to the APAIS data, but they used a more complicated model structure (mixed logit) and different types of weighting factors (e.g., interview effort). They did not formally test for statistically significant differences between the weighted and unweighted estimates of WTP in their shore fishing case study.

A number of studies have estimated angler WTP for groupers and red snapper in the southeastern United States. Carter and Liese (2012) estimated that anglers were willing to pay between $25 and $80 (in 2003 dollars) to keep an additional grouper, depending on how many fish they had already kept on a trip. Their comparable estimates for red snapper ranged from $20 to $62. These results are considerably higher than the estimates reported in our study (Table 4). However, the analysis by Carter and Liese (2012) was based on stated preference data, which tend to generate relatively higher WTP estimates than revealed preference data such as the data we used in our site-choice demand analysis (Johnston et al., 2006). Haab et al. (2012) pre-

Table 3

The effect of incorporating survey weights into a conditional logit model of angler site-choice is shown below for 4 different versions of the model. The unweighted version included no adjustments for survey weights. The other 3 versions incorporated weights on the basis of the ratio of the proportion of total angler trips by zone to sample proportions. Different weighted versions of the model, with mean values or the lower or upper limit of 95% confidence intervals of the total proportion of estimated trips, were used to estimate parameter values with a weighted exogenous sampling maximum likelihood function (WESML). Estimated parameters (with standard errors of the mean in parentheses) are given for the independent variables. Data used in analyses are from the National Marine Fisheries Service's Angler Access Point Intercept Survey for trips in the Gulf of Mexico in 2009 that targeted groupers (*Epinephelus* spp., *Hyporthodus* spp., or *Mycteroperca* spp.) or red snapper (*Lutjanus campechanus*).

		WESML		
Variable	Unweighted	Mean	Lower limit	Upper limit
Travel cost (2009 dollars)	−0.060 (0.002)	−0.056 (0.002)	−0.067 (0.003)	−0.054 (0.002)
Log of number of sites per zone	0.251 (0.099)	0.242 (0.092)	0.571 (0.142)	0.063 (0.085)
Grouper harvest rate per angler (number of fish) caught and kept)	0.784 (0.189)	0.819 (0.174)	0.543 (0.280)	0.829 (0.168)
Red snapper harvest rate per angler (number of fish caught and kept)	0.471 (0.099)	0.603 (0.091)	0.303 (0.126)	0.748 (0.088)
Log likelihood function	−914.42	−942.68	−838.64	−971.55
Number of zones in model	28	28	16	28

sented the most recent study that is most comparable to our study. Their estimates of angler WTP for red snapper and groupers calculated with APAIS data from 2000 in the Gulf of Mexico were also much higher than our estimates. On the basis of the most comparable model (conditional logit), they estimated angler WTP for an additional red snapper at $123 and for an additional grouper at $91 (in 2000 dollars). At least part of the differences in WTP estimates for groupers and red snapper between the Haab et al. (2012) study and our study likely was due to the inclusion of the for-hire mode (with higher associated travel costs) and the opportunity cost of time in their model. However, it is difficult to tell how much those inclusions contributed to the differences in the WTP estimates without a more detailed comparison of the models.

This study was based on the private-boat angler's choice across boat launch sites. However, anglers who launch from either a fixed boat slip or a boat storage facility will not regularly choose to launch from other places. Unfortunately, the APAIS data does not identify whether a boat was launched from a trailer, a slip, or a fixed dock. In the case of the APAIS data used in our study, more than 64% of the intercept sites along the Gulf of Mexico where anglers were interviewed in 2009 were only boat ramps with no associated boat slips or boat storage areas. The majority of anglers included in our study trailer their boats and, therefore, have a choice regarding what launch site they use. The potential limitation of the standard site-choice model with regard to choice of boat launch site should be explored in the context of future work, but it does not affect our results from the use of APAIS weights in our site-choice models.

Conclusions

In this study, different versions of a site-choice model for analysis of fishing site choices in the Gulf of Mexico in 2009 were estimated with recently available sampling weights from the APAIS survey in order to illustrate how site-choice models can incorporate a complex sample design and reduce potential biases in estimation. Model results indicate that the addition of sampling weights affected the estimated parameters for historic catch-and-keep rates. The difference

Table 4

The mean willingness to pay (in dollars) per angler for an additional fish caught and kept (with standard errors) estimated for groupers (*Epinephelus* spp., *Hyporthodus* spp., or *Mycteroperca* spp.) and red snapper (*Lutjanus campechanus*) and based on a conditional logit model of angler preferences for fishing sites in the Gulf of Mexico in 2009. The lower limit and upper limit of the 95% confidence intervals of mean willingness to pay also are shown. Four versions of the model were used to estimate willingness to pay. The first version did not account for survey weights (unweighted). The other versions of the model were adjusted with weights on the basis of the ratio of the proportion of total angler trips to sample proportions and with a weighted exogenous sampling maximum likelihood function (WESML). In the second version, the total population proportion of trips was evaluated at the mean, in the third version it was evaluated at the lower limit of the 95% confidence interval, and in the fourth version, it was evaluated at the upper limit of the 95% confidence interval.

Model	Mean	Standard error	Lower limit	Upper limit
Groupers				
Unweighted	$13.01	2.95	$7.22	$18.80
WESML (mean)	$14.67	2.91	$8.96	$20.39
WESML (lower limit)	$8.07	3.95	$0.33	$15.81
WESML (upper limit)	$15.32	2.89	$9.65	$20.98
Red snapper				
Unweighted	$7.81	1.61	$4.66	$10.97
WESML (mean)	$10.81	1.59	$7.68	$13.93
WESML (lower limit)	$4.51	1.84	$0.90	$8.11
WESML (upper limit)	$13.81	1.60	$10.67	$16.95

in parameter estimates translated into different WTP amounts for an additional fish caught and kept. However, the differences in angler WTP estimated between the weighted and unweighted versions of the model were not statistically different. This finding indicates that, although sampling weights can be used to correct for issues of endogenous stratification in on-site sampling and to reduce bias in parameter estimates, the bias in the related angler WTP measures may not be severe in the APAIS data used in our study. Whether this holds for other APAIS samples is an open question. For researchers who estimate recreational site-choice models with APAIS data, it is advisable to compare results produced with and without the APAIS sampling weights developed by NMFS. We have shown how to incorporate APAIS sampling weights into site-choice models using data from a popular recreational fishery in the Gulf of Mexico. The question of whether weighted or unweighted results should be used in future applications will have to be determined on a case-by-case basis.

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Aspects of the reproductive biology of the North Pacific giant octopus (*Enteroctopus dofleini*) in the Gulf of Alaska

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Abstract—In 2011, octopuses in the Gulf of Alaska were removed from the "other species" group and are now managed by the North Pacific Fishery Management Council as a complex that includes all octopus species within this region. Management of this complex includes the specification of annual catch limits and overfishing limits. Understanding the life history of octopuses is important for establishment of appropriate management strategies. The North Pacific giant octopus (*Enteroctopus dofleini*) is the most abundant octopus species found on the continental shelf and dominates the commercial catch of octopuses within the Gulf of Alaska. Specimens of the North Pacific giant octopus were obtained from charter operations, commercial fishermen, and scientific surveys within the Gulf of Alaska. This species has a protracted reproductive cycle and peak spawning occurs from winter to early spring months. In the Gulf of Alaska, this species matures at weights from 10 to 20 kg; weight at 50% maturity is 13.7 kg (95% confidence interval [CI]=12.5–15.5 kg) for females and 14.2 kg (95% CI=12.6–15.9 kg) for males. Estimates of fecundity for this species range from 41,600 to 239,000 eggs per female and average fecundity is estimated at 106,800 eggs per female. Fecundity was positively related to the weight of the female (n=33, P<0.001). Determination of reproductive parameters is necessary to assess the vulnerability of octopuses within this region to overfishing and to establish appropriate management strategies for this species group within the Gulf of Alaska.

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Recent changes in the management of commercial fisheries in Alaska have included increased monitoring and regulation of several species groups that are not directly targeted but are incidentally taken in fisheries managed by federal and state agencies. An octopus management complex, with its own annual catch limits and overfishing limits was created by the North Pacific Fishery Management Council in 2011 in both the Bering Sea and Gulf of Alaska regions. There is no fishery that directly targets this complex, but octopuses are taken as bycatch in trawl, longline, and pot fisheries throughout Alaska—the majority of catch coming from pot fisheries targeting Pacific cod (*Gadus macrocephalus*) (Sagalkin and Spalinger, 2011; Conners et al., 2012). At least 8 species of octopuses are found within the Gulf of Alaska and are members of this complex, but the North Pacific giant octopus (*Enteroctopus dofleini*) is the most abundant species in continental shelf waters within this region (Jorgensen, 2009; Conners et al., 2012). This species dominates incidental catch of octopuses in commercial fisheries managed by the North Pacific Fishery Management Council and by the state of Alaska (Conners et al., 2012).

The North Pacific giant octopus is found throughout the North Pacific Ocean from Japanese waters to the Aleutian Islands, Bering Sea, and the Gulf of Alaska and in coastal waters in the eastern Pacific as far south as Southern California (Jorgensen, 2009). It is the largest species of octopuss in the world, reaching a maximum size of around 50 kg (Roper et al., 1984). The life expectancy of this species appears to be 4.5 to 5 years on the basis of aquarium studies conducted within the Puget Sound region of Washington state. Like most incirrate octopuses, this species is semelparous and, therefore, females die after a single batch of eggs hatch (High, 1976). This species is estimated to have up to 100,000 eggs per female and a large number of planktotrophic larvae (Kanamaru, 1964; Sato, 1996). Hatchlings have been found to be about 3.5 mm in length (Kubodera, 1991). It has been assumed that females of this species have the ability to store sperm (Kanamaru, 1964), and this phenomenon has been documented in an aquarium setting (Gabe, 1975).

The life history of the North Pacific giant octopus has been examined in other parts of their range, particularly in Japanese waters (Kanamaru, 1964; Kanamaru and Yamashita, 1967; Sano et al., 2011) and in Canadian waters near British Columbia (Gabe, 1975; Robinson, 1983). However, there is a lack of information on the biology and ecology of all octopus species in the Gulf of Alaska. These data are needed to determine bycatch limits and management strategies for octopus species within this region. The objective of this study was to examine the reproductive biology of the North Pacific giant octopus within the Gulf of Alaska, specifically to determine the seasonality of reproduction, size at maturity, and fecundity of this species in this region.

Materials and methods

Maturity stages

Specimens of the North Pacific giant octopus were obtained from the Gulf of Alaska from commercial fishermen during February 2010, the Alaska Fisheries Science Center bottom-trawl survey of the Gulf of Alaska in the summer of 2011, and charter operations near Kodiak Island during the period from May 2010 to December 2011. Octopuses were identified as North Pacific giant octopus on the basis of characteristics presented in Jorgensen (2009). Charter operations occurred within Chiniak Bay and specimens from the bottom-trawl survey were collected at 4 locations in the western and central Gulf of Alaska (Fig. 1). The exact location of specimens donated from commercial fishermen is unknown, but specimens were captured in the Gulf of Alaska near Kodiak Island. A total of 154 specimens were collected. These specimens, including both females (n=71) and males (n=83), ranged in size from 1.2 to 25.2 kg.

All sampled octopuses were weighed, their sex was determined, and their reproductive tracts were removed and weighed. The weight and diameter of each gonad was measured, and the condition of the reproductive tract was noted. Female reproductive structures were examined for the presence of spermatophores or other evidence of mating activity. A 3-stage maturity classification system was developed as part of this study for both male and female North Pacific giant octopuses on the basis of characteristics of the reproductive tract and the presence or absence of well-developed eggs or spermatophores.

On the basis of this classification system, which is a modification of the classification system presented in a report by the Fisheries and Oceans Canada,[1] each specimen was assigned a maturity stage of 1 (immature), 2 (maturing), or 3 (mature). A female was considered immature (stage 1) if it possessed an ovary that was small (diameter ≤65 mm) and white and had eggs not easily visible; maturing (stage 2) if its ovary was larger (diameter 69–90 mm) and had small, white, or pale-yellow eggs; and mature (stage 3) if its ovary was large (≥100 mm) and yellow and the majority of eggs were large (≥5 mm), dark, and yellow. A male was considered immature (stage 1) if its reproductive tract was transparent and undifferentiated, maturing (stage 2) if its reproductive tract contained well-developed accessory structures but had no fully developed spermatophores, and mature (stage 3) if visible and fully developed spermatophores were present within its reproductive tract.

For all specimens, all or part of the gonad was preserved. Thin sections of these tissues were embedded in paraffin and stained through the use of standard histological techniques (Sheehan and Hrapchak, 1980). These sections were used to verify the visual assessment of the gonad and to measure egg size in female octopuses.

Size at maturity

Octopus size at maturity was assessed because aging of cephalopods is difficult (Guerra et al., 2010) and there is no established procedure for aging of the North Pacific giant octopus. Octopus lengths are difficult to measure consistently because of the fluidity of octopus movement and differences found in measurement of live (mantle contracting) and dead (mantle not contracting) octopuses. The weight at maturity of octopuses is generally assessed in studies of octopus biology (Hernández-García et al., 2002). A gonadosomatic index (GSI) was calculated for each octopus sampled by dividing the wet weight of the reproductive tract by the total wet weight of the octopus. A variety of GSI-type indices have been used in octopus reproductive studies (Perez and Haimovici, 1991; Cortez et al., 1995; Quetglas et al., 2005). For this study, the simplest method was chosen. Gonadosomatic index values for both males and females were examined across all size ranges to see if there was a pattern in development.

Weight at 50% maturity (W_{50}) was estimated by fitting the data to the following logistic equation:

$$Y = 1 / (1 + e - (a + bX)),$$

where Y = the proportion of specimens that were mature at weight X;
a and b = parameters of the model; and
X = the weight of the octopus.

This model was chosen because of its wide application in fisheries research and because logistic models are prevalent in recent octopus research (Quetglas et al., 2009; Šifner and Vrgoć, 2009). Parameters were fitted to the logistic equation with a generalized linear

[1] Fisheries and Oceans Canada. 2003. Pacific regions: 2003 experimental harvest guidelines, octopus by dive, January 16, 2003 to July 31, 2003, 21 p. [Available from http://www.dfo-mpo.gc.ca/Library/315447.pdf.]

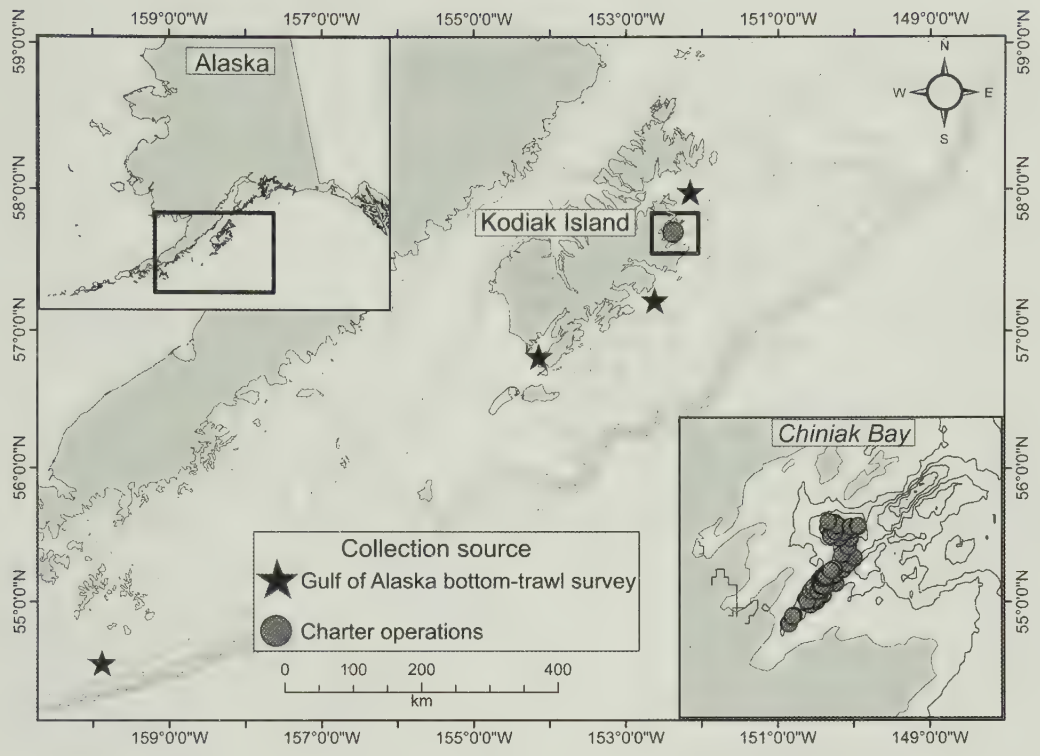

Figure 1

Map of sampling locations where North Pacific giant octopuses (*Enteroctopus dofleini*) were collected during 2010–2011 in the central and western Gulf of Alaska from both charter operations and the bottom-trawl survey conducted by the Alaska Fisheries Science Center in the Gulf of Alaska. The inset on the upper left of the figure shows the location of the sampling area in relation to the state of Alaska. The inset on the lower right of the figure provides greater detail on the locations where octopuses were sampled during charter operations in Chiniak Bay.

model, and the equation was solved for Y=0.5 to yield W_{50}. Bootstrapping methods were used to estimate confidence intervals and the variance of W_{50} (Efron and Tibshirani, 1993). These statistical tests were completed with TIBCO Spotfire S+ statistical software,[2] vers. 8.2 (TIBCO Software, Palo Alto, CA). In addition, for maturing and mature female specimens, the histological sections were examined to determine the longest ova: 5 large ova in each section were measured with a micrometer, and the largest was determined to be the maximum ova length (MOL).

Seasonality

Seasonality of reproduction in this species was examined by noting the presence or absence of spermatophores and well-developed, yolked (stage-3) ovarian eggs during each season of the year. Seasons were defined on the basis of daily measurements of sea-surface temperatures in Chiniak Bay taken at the Kodiak Fisheries Research Center: spring had rising temperatures (April–June), summer had rising temperatures to a maximum (July–September), fall had falling temperatures (October–December), and winter temperatures were steady and coldest (January–March). The GSI values for maturing and mature North Pacific giant octopuses were averaged by season to determine a mean seasonal GSI values for males and females. This seasonal examination of gonad growth was limited to individuals with developing reproductive tracts and, therefore, did not include immature specimens that may remain immature for a period of years. The mean MOL also was determined for each season of the year. A one-way analysis of variance (ANOVA) was used to test for significant differences in GSI and female MOL values during each season of the year. If results were found to significantly differ, the Tukey honest significant difference (HSD) test was used to compare the GSI and MOL values between individual seasons. The ANOVA and Tukey HSD tests were completed with R statistical software, vers. 2.15.1 (R Core Team, 2012).

[2] Reference to trade names or commercial companies is for identification purposes only and does not imply endorsement by the National Marine Fisheries Service, NOAA.

Fecundity

Fecundity was estimated for stage-2 and stage-3 females with developing eggs within their ovaries. The fecundity of stage-1 females was not examined because of a lack of development of some stage-1 females and the small size of eggs found within the ovaries of these females. For each female classified as stage 2 or 3, a sample that weighed between 1.5 and 3 g was removed from the ovary and all eggs within it were counted with a dissecting microscope. This subsample was used to estimate the total number of eggs within an ovary by applying a gravimetric approach. With this approach, the estimated individual egg weight was used in combination with the weight of the whole ovary to estimate the number of eggs found within each ovary. The relationship between total body weight and fecundity for females was plotted, and an exponential regression line was fitted to the data with R software.

Results

Size at maturity

Female GSI values ranged from <0.001 to 0.083 and overlapped between stages (Fig. 2A). Female North Pacific giant octopuses had MOL values ranging from 0.1 to 8.1 mm. Stage-1 females had MOLs that were <2 mm, stage-2 females had MOLs ranging from 2.6 to 7.8 mm, and stage-3 females had MOLs ranging from 3.6 to 8.1 mm. MOLs of stage-2 females were predominately ≤5.0 mm with one exception, and most MOLs of stage-3 females were >5.0 mm.

Male North Pacific giant octopuses had GSI values that ranged from <0.003 to 0.082 (Fig. 2B). The overlap in GSI values for all stages is a reflection of the large size range of stage-2 males. Many stage-2 males possessed reproductive structures that were well-developed and large but lacked spermatophores. Because spermatophores are necessary for successful mating, these male octopuses were determined to be stage 2. They all appeared to be healthy— without any obvious signs of senescence (reduced body condition or uncoordinated body movements), and we assumed that they had been captured before maturation rather than after mating.

Size at maturity was highly variable for both sexes, but it was particularly so for male North Pacific giant octopuses. The smallest mature male was 7.5 kg, and the largest immature male was 22.5 kg (Fig. 2A). The smallest mature female was 9.2 kg, and the largest immature female was 18.0 kg (Fig. 2B). North Pacific giant octopuses <10 kg tended to be immature, but male and female members of this species in the size range of 10–20 kg were found at all 3 maturity stages. Weight at 50% maturity for males and females fitted the logistic model and was found to be near 14 kg for both sexes, but it was highly variable between individuals

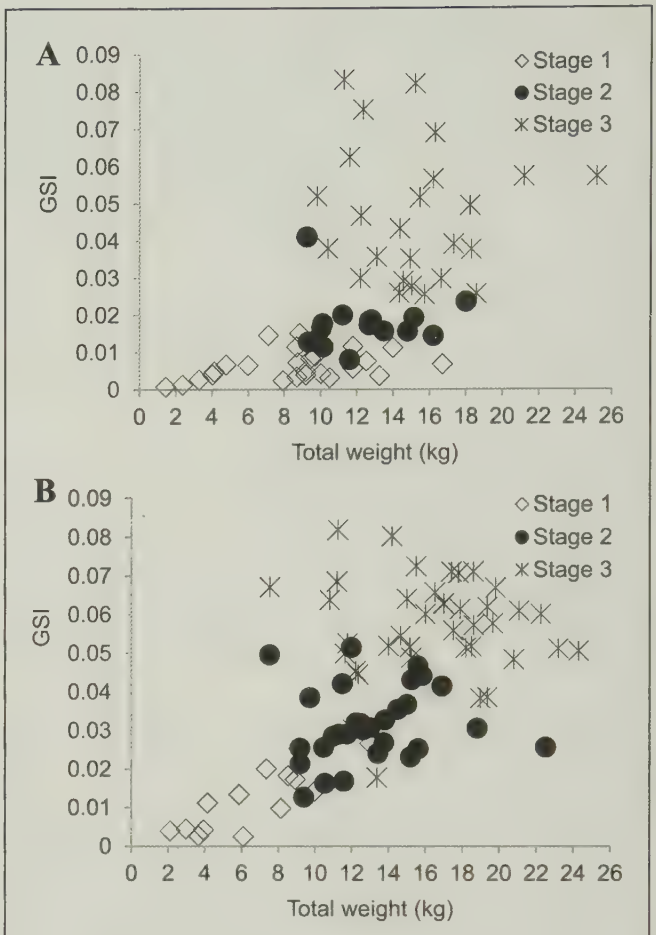

Figure 2

Gonadosomatic index (GSI) by total weight for (**A**) all female specimens and (**B**) all male specimens of the North Pacific giant octopus (*Enteroctopus dofleini*) collected from the central and western Gulf of Alaska in 2010–2011. Values are shown for 3 maturity stages: immature (stage 1), maturing (stage 2), and mature (stage 3).

(males, W_{50}=14.2 kg, P<0.001, 95% confidence interval [CI]=12.6–15.9 kg; females, W_{50}=13.7 kg, P<0.001, 95% CI=12.5–15.5 kg; Fig. 3).

Seasonality

Mature males and females were observed within each sampling season (Table 1). The seasonal GSI for females peaked in the winter season, was at a minimum during spring months, and increased steadily throughout the summer and fall (Fig. 4A). The seasonal GSI values were significantly different between seasons (P<0.001); significant differences were found between 3 seasonal comparisons: spring and fall (P=0.004); winter and spring (P=0.001); and winter and summer (P=0.011). These results indicate a significant increase

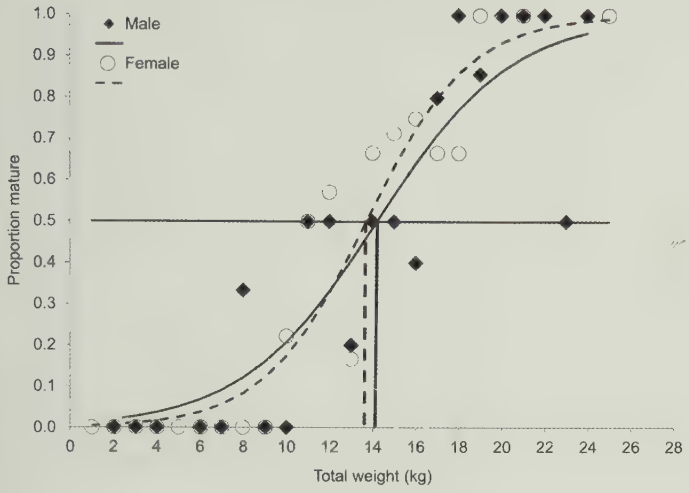

Figure 3

The proportion mature as a function of body weight of male (diamonds) and female (circles) North Pacific giant octopuses (*Enteroctopus dofleini*) collected from the central and western Gulf of Alaska in 2010–11. The vertical lines indicate the weights at which 50% of the males (solid line) and females (dotted line) were mature. The curved lines are the logistic curves fitted to the data for males (dotted line) and females (solid line).

in GSI values during the fall and winter months in comparison with the spring and summer months. The number of samples used in this analysis was small, but these results provide evidence that reproduction for this species is weakly synchronous with a peak in egg development in the coldest months of the year.

A similar pattern is evident in the MOL data, with peak egg size occurring in the fall and winter months. The seasonal MOL values also were significantly different between seasons (P=0.003), with significant differences found between the spring and fall (P=0.002) and the summer and spring (P=0.033). This evidence indicates that the majority of females lay eggs in late winter in this region. Spermatophores or other evidence of mating was not found within female octopuses during any of the sampling periods. These patterns are not found in the males of this species. Male GSI values were highest in winter and spring but did not vary as much as the female values throughout the year (Fig. 4B). The seasonal GSI values for males were not found to be significantly different between seasons (P=0.452).

Fecundity

Estimated fecundity of mature and maturing North Pacific giant octopuses ranged from 41,600 to 239,000 eggs per female and mean fecundity was 106,800 eggs per female (n=33, standard error=7900). There was a positive relationship between fecundity and total weight of female North Pacific giant octopuses, but there was a high amount of individual variability in fecundity (Fig. 5). Exponential regression of fecundity on total weight of female octopuses was highly significant (P<0.001), but the predictive value of this relationship was low, as indicated by a low coefficient of multiple determination (R^2=0.471).

Discussion

Size at maturity

We found that North Pacific giant octopuses in the Gulf of Alaska mature at a size of about 14 kg—a finding similar to results from another study in Japanese waters, a study in which females of this species were estimated to mature at 10–15 kg and males at 7–17 kg (Kanamaru and Yamashita, 1967). Recent research indicated size at maturity of this species is smaller within the Bering Sea, with a calculated W_{50} of 12.8 kg for females and 10.8 kg for males (Brewer[3]). In British Columbia waters, male North Pacific giant octopuses were also found to mature at around 12.5 kg and at a smaller size than females (Robinson, 1983), but that study did not include mature females. Although our value for weight at 50% maturity was larger for males than for females, male size at maturity was more variable and there were larger

[3] Brewer, R. 2012. Personal commun. Univ. Alaska Fairbanks, Unalaska, AK 99685.

Table 1

Frequency of maturity stage observed during each season of the year for male and female North Pacific giant octopuses (*Enteroctopus dofleini*) captured in the western and central Gulf of Alaska in 2010–11. The spring season was from April to June, the summer season was from July to September, the fall season was from October to December, and the winter season was from January to March.

Season	Immature	Maturing	Mature
Male			
Spring	2	2	13
Summer	8	3	9
Fall	1	11	5
Winter	3	14	12
Female			
Spring	8	4	2
Summer	10	7	4
Fall	2	4	11
Winter	7	2	9

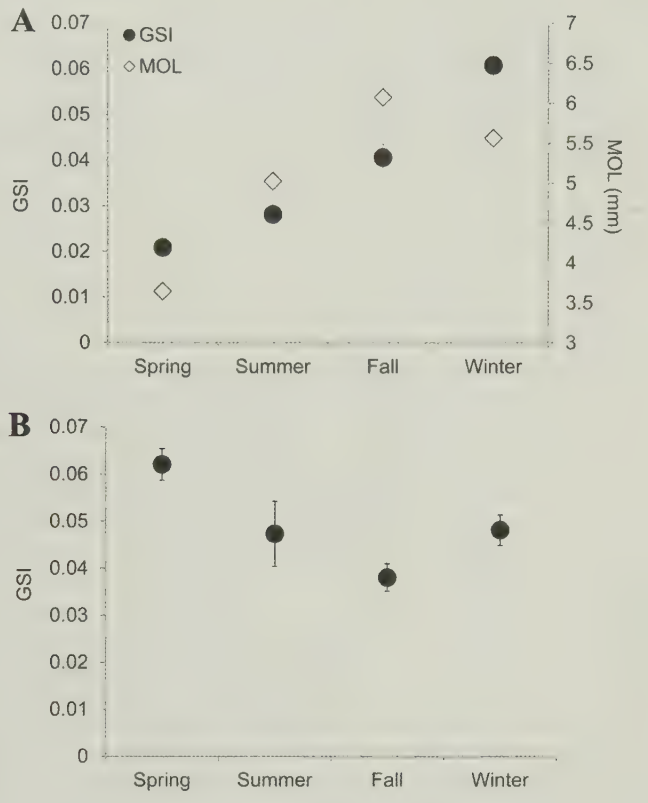

Figure 4

(**A**) Gonadosomatic index (GSI) and maximum ova length (MOL) by season for maturing and mature female specimens of North Pacific giant octopuses (*Enteroctopus dofleini*) and (**B**) GSI by season for maturing and mature male North Pacific giant octopuses. Specimens were collected from the central and western Gulf of Alaska in 2010–2011. Error bars indicate standard error of the mean.

fractions of both small mature males and large immature males. Growth and maturity of this species appear to be quite variable, and regional differences in size at maturity may reflect differences in environmental conditions, food availability, or genetic differences between stocks.

Seasonality

The life history of the North Pacific giant octopus in the Gulf of Alaska is similar to that in Japanese and Canadian waters of the North Pacific Ocean, but the timing of reproductive events is different for members of this species in the Gulf of Alaska. In Japanese waters, this species has been reported to mate during summer and early fall (July–October) and to lay eggs or spawn during fall and winter (October–January) with a 2-month lag between mating and spawning (Kanamaru, 1964). Another study in Japanese waters found that the spawning season occurred in early summer (Sano et al., 2011). In the Bering Sea during a recent study, female development was most advanced in the fall months and male development did not show a seasonal pattern (Brewer[3]). Our study indicates that peak spawning in the Gulf of Alaska occurs during the winter months (January–March). The development of male gonads indicates that mating may peak earlier in the spring months.

Results from our study indicate that mating occurs predominately in the spring and summer months but may be very protracted and occur throughout the year. It is assumed that females of this species are capable of storing sperm. This phenomenon has been documented in an aquarium study of North Pacific giant octopuses in British Columbia (Gabe, 1975) and has been observed in several other octopus species (Joll, 1976; Perez et al., 1990). The seasonal differences in the timing of peak GSI values between male and female North Pacific giant octopuses indicate that storage of sperm may occur in octopuses in the Gulf of Alaska between spring mating and winter spawning. Females of this species appear to lay eggs predominately during winter, although the presence of mature females throughout the year indicates that some spawning may occur during other periods of the year.

This species is found throughout the North Pacific Ocean, but there is little information available about its seasonal movements in Alaska waters. It is, therefore, difficult to interpret how the habitat of these octopuses differs during periods of the year when octopuses are mating and spawning. Japanese studies indicate that North Pacific giant octopuses in waters off of the coast of Hokkaido, Japan, move to deeper waters in summer to mate and move into shallower waters to spawn (Kanamaru, 1964). However, no evidence of a seasonal or directed migration for this species has been found in studies of this species in British Columbia (Hartwick et al., 1984) and south central Alaska (Scheel and Bisson, 2012). Long-term tagging studies are needed to obtain a more complete understanding of the migratory pattern of this species.

Another complicating factor that may contribute to the variability in both size at maturity and seasonality of the reproduction of the North Pacific giant octopus is the complicated stock structure and phylogenetic relationships of this species throughout its range. Three subspecies have been identified on the basis of large geographic ranges and morphological characteristics: *Enteroctopus dofleini dofleini* (far western North Pacific Ocean), *E. dofleini apollyon* (waters near Japan, Bering Sea, Gulf of Alaska), and *E. dofleini martini* (eastern part of range [Pickford, 1964]). A recent genetic study (Toussaint et al., 2012) indicated the presence of a cryptic species of North Pacific giant octopus in Prince William Sound, Alaska, and raises additional questions about the stock structure of this species. More data are needed to define the stock structure of this

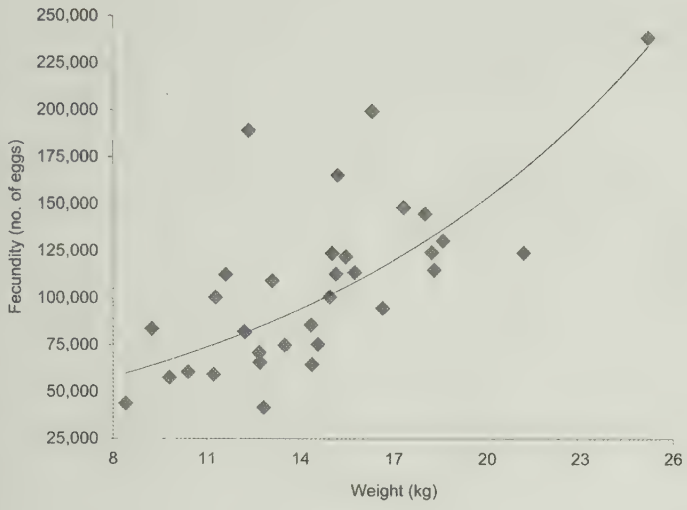

Figure 5

Fecundity, measured as number of eggs per female, by total body weight for North Pacific giant octopuses (*Enteroctopus dofleini*) classified as stage 2 (maturing) and stage 3 (mature) and collected from the central and western Gulf of Alaska during 2010–11. The exponential regression line is presented.

species in Alaska waters, and additional data may aid in reduction of the variability of estimates of reproductive parameters.

Fecundity

Fecundity in the Gulf of Alaska was found to be greater than previously reported for this species, although results of this study did have some overlap with values reported from studies in Canada and Japan. The fecundity of this species in Japanese waters has been estimated to be from 30,000 to 100,000 eggs per female (Kanamaru, 1964; Mottet, 1975; Sato, 1996). Gabe (1975) estimated a female in captivity in British Columbia had a realized fecundity of 35,000 eggs. We found higher mean fecundity values of 106,800 eggs per female in the Gulf of Alaska and these values had a significant positive relationship with the overall weight of the female. The values of fecundity from our study and the Japanese studies were estimates of potential fecundity and did not account for any losses that may have occurred because of either atresia or eggs not released by a female. Realized or actual fecundity will be smaller than these values, although, for a semelparous species, the difference may not be significant. The high fecundity of the North Pacific giant octopus, in combination with its short life span and relatively fast growth rate (High, 1976), indicates a potentially high rate of population productivity. However, commercial harvest should be managed conservatively because of a lack of knowledge about a variety of population parameters for this species in Alaska waters, including recruitment rates, abundance, movement, and natural mortality.

Conclusions

This study improves our understanding of the reproductive biology of the North Pacific giant octopus in the Gulf of Alaska. The weight at maturity of about 14 kg for both males and females is larger than values reported for this species within other regions of the North Pacific Ocean. The seasonality of reproduction also appears to be somewhat different in the Gulf of Alaska as well, where reproductive events occur a few months later than they do in Japanese waters. We also found higher fecundity for this species in this region. These data will aid in the management of this species complex and will be particularly important in guiding management decisions if fisheries for octopuses develop within the Gulf of Alaska.

Acknowledgments

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Regional differences in the age and growth of red snapper (*Lutjanus campechanus*) in the U.S. Gulf of Mexico

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Abstract—In the U.S. Gulf of Mexico (GOM), red snapper (*Lutjanus campechanus*) are managed as a unit stock, although the stock is assessed as subunits east and west of the Mississippi River. Differences were examined between management subunits by comparison of the size and age structure and growth rates of red snapper among recreational catches from 6 regions of the GOM: South Texas, North Texas, Louisiana, Alabama, Northwest Florida, and Central Florida. In all of these regions, red snapper sampled in 2009 and 2010 were small and predominantly from age classes that represented the strong recruitment for the year classes of 2004–06. As such, our data indicate a highly truncated age structure with few fish older than 6 years. Demographic differences in size, age, and growth parameters were found. Small (≤550 mm in total length), fast-growing individuals dominated the catches in South Texas and the regions of Florida, whereas larger, slower-growing fish represented the majority of catches in Alabama and Louisiana. The potential mechanisms affecting observed demographic variation include environmental differences, fishing pressure, habitat preference, and management regimes; however, no definitive conclusion about cause and effect can be made. The combination of demographic differences between regions and consistent occurrence of the strong year classes GOM-wide supports recent conclusions that red snapper form a metapopulation of semi-isolated assemblages in the GOM.

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The stock of red snapper (*Lutjanus campechanus*) in the U.S. Gulf of Mexico (GOM) has been exploited since the mid-1800s, and the red snapper fishery is still one of the most economically important reef fish fisheries in the GOM. This fishery has multimillion-dollar commercial and recreational sectors and is affected by juvenile mortality that results from bycatch in the shrimp trawl fishery. Since the early 1990s, GOM red snapper have been managed intensely as a single-unit stock through both frequent management intervention and significant catch constraints. However, this stock has been declining since the 1970s and was overfished for most of the 1990s and 2000s (Goodyear, 1997; SEDAR[1]; Porch, 2007; GMFMC[2]; NMFS[3]). The current management policy has set a rebuilding plan for stock recovery by 2032, and the most recent stock assessments indicate that, although this stock is overfished, overfishing is no longer occurring (GMFMC[4]; SEDAR[5]).

[1] SEDAR (SouthEast Data, Assessment, and Review). 2005. Stock assessment report of SEDAR 7: Gulf of Mexico red snapper, 480 p. [Available from http://www.sefsc.noaa.gov/sedar/.]

[2] GMFMC (Gulf of Mexico Fishery Management Council). 2007. Final Amendment 27 to the Reef Fish Fishery Management Plan and Amendment 14 to the Shrimp Fishery Management Plan, 480 p. [Available from Gulf of Mexico Fishery Management Council, 2203 North Lois Ave., Suite 1100, Tampa, FL 33607.]

[3] NMFS (National Marine Fisheries Service). 2012. Annual report to Congress on the status of U.S. fisheries—2011, 20 p. [Available from NMFS, NOAA, 1315 East-West Hwy., Silver Spring, MD 20910.]

[4] GMFMC (Gulf of Mexico Fisheries Management Council). 2010. Final regulatory amendment to the Reef Fish Fishery Management Plan to set total allowable catch for red snapper, 98 p. [Available from Gulf of Mexico Fishery Management Council, 2203 North Lois Ave., Suite 1100, Tampa, FL 33607.]

[5] SEDAR (SouthEast Data, Assessment, and Review). 2013. SEDAR 31—Gulf of Mexico red snapper stock assessment report, 1103 p. [Available from http://www.sefsc.noaa.gov/sedar/.]

Although red snapper in the GOM currently are managed as a single unit stock, separate stock assessments have been conducted since 2004 for subunits east and west of the Mississippi River (SEDAR[1]). Under the single-unit-stock hypothesis, no significant differences are assumed in population structure (genetics and population demographics) across the GOM. The single-unit-stock assumption has been supported by genetic analysis (Camper et al., 1993; Gold et al., 1997; Gold et al., 2001; Saillant et al., 2010), by the potential for high larval dispersal through hydrodynamic transport (Johnson et al., 2009), and by the capacity of adult red snapper to move great distances (>100 km) and, therefore, to potentially maintain mixing rates (Patterson et al., 2001a). Also, in the past 20 years, 2 strong year classes (1989 and 1995) were found to dominate catches GOM-wide (Allman and Fitzhugh, 2007), strengthening the single-unit-stock hypothesis.

In the recent decade, however, numerous studies have highlighted spatial differences in age, growth, and reproductive demographics between eastern and western red snapper (Allman et al., 2002; Fischer et al., 2002, 2004; Jackson et al., 2007). Studies of the population structure of red snapper genetics and movement indicate that GOM fish form a metapopulation of semi-isolated, subpopulations (Saillant and Gold, 2006; Gold and Saillant, 2007; Patterson, 2007), and recent genetic evidence indicates that the Caribbean red snapper (*L. purpureus*) and the red snapper (*L. campechanus*) may not be separate species (Gold et al., 2011; Gomes et al., 2012). Examination of otolith microchemistry also has shown region-specific natural tags or *elemental signatures*, which have been used to identify nursery sources, subpopulations, and to provide evidence of stock mixing across the GOM (Patterson et al., 2008; Nowling et al., 2011; Sluis et al., 2012).

In this study we sought to complement similar measures reported by Fischer et al. (2004) for a previous study and to expand upon the area that they studied in order to encompass more regions. The updated information from our study is important for identification of region-specific trends and changes in the red snapper fishery. Our objectives were 1) to examine the size structure, growth rates, and size at age of red snapper in the GOM to elucidate trends in demographic differences noted in the most recent stock assessments (SEDAR[5]), 2) to expand the comparison to incorporate red snapper off Florida, and 3) to identify region-specific trends in the recreational red snapper fishery.

Materials and methods

Red snapper were sampled from recreational hook-and-line fisheries (head boats and charter boats) in 6 regions of the GOM during 2009 and 2010 (Fig. 1) and were identified by using characteristics from Hoese and Moore (1998). In each region and year, a maximum of 200 fish were sampled from the daily catches of recreational vessels. Fish were selected haphazardly while the vessels offloaded. Sample size varied with trip size, and the number of vessels sampled in each region was dependent upon the size of the regional fleet. To obtain samples representative of the fishery in each region and to maximize the number of vessels and trips sampled, collection occurred during a period of 3–5 days each year. Samples were not selected from the commercial fishery because of the market preference for fish close to 330 mm in total length. During 2010, recreational fisheries in Alabama and Louisiana were not sampled because these fisheries were closed as a result of the Deepwater Horizon Oil Spill. Louisiana samples were supplemented in 2010 with fish collected by hook and line from 2 oil platforms, which are sites routinely fished by recreational and commercial fishermen, in the GOM about 110 km south of the Louisiana coast. For all fish collected, morphometric measurements (natural total length [TL] in millimeters and total weight [TW] in kilograms) were recorded, sex was determined by macroscopic examination of gonads (when possible), and sagittal otoliths were removed, rinsed, and stored in coin envelopes until processed.

The left sagittal otolith was sectioned in a transverse plane following the methods of Cowan et al. (1995) with a Hillquist Thin Section Machine, Model 800[6] (Hillquist Inc., Denver, CO), equipped with a diamond-embedded wafering blade and precision grinder. Otolith sections (0.2-mm thick) were read under a dissecting microscope with transmitted light and a polarizing light filter at a magnification from 20× to 64×. Counts of opaque annuli were made along the dorsal margin of the sulcus acousticus from the core to the proximal edge (Wilson and Nieland, 2001). Edge condition of the dorsal margin was coded according to Beckman et al. (1988). Annuli were counted by 2 independent readers without knowledge of date and location of capture or access to morphometric data of the specimens. When initial counts disagreed, annuli were counted a second time. In instances where a consensus between the 2 readers could not be reached, the annulus counts from the more experienced reader were reported. Precision between readers was evaluated with the coefficient of variation (CV), index of precision (D) (Chang, 1982), and average percent error (APE) (Beamish and Fournier, 1981). Ages of red snapper were estimated from the number of opaque annuli, assumed birthdate, and capture date, by following the equation described by Wilson and Nieland (2001):

$$Age\ (\text{days}) = -182 + (annulus\ count \times 365) + ((m - 1) \times 30) + d, \quad (1)$$

where m = the ordinal number (1–12) of month of capture; and

[6] Mention of trade names or commercial companies is for identification purposes only and does not imply endorsement by the National Marine Fisheries Service, NOAA.

Figure 1

Map of the Gulf of Mexico showing the 6 regions where red snapper (*Lutjanus campechanus*) were collected for age and growth analysis from recreational catches in 2009 and 2010. The 6 regions were South Texas, North Texas, Louisiana, Alabama, Northwest Florida, and Central Florida.

d = the ordinal number (1–31) of the day of the month of capture.

It was assumed that annulus formation began on 1 January, and the periodicity of opaque zone formation was verified with edge analysis (Wilson and Nieland, 2001; Fischer et al., 2004). To account for the uniform birthdate of 1 July, 182 days were subtracted from each age estimate.

Analysis of variance (ANOVA) was used to compare mean TL, TW, and age among regions, and all statistical analyses were performed with SAS analytics software, vers. 9.1.3 (SAS Institute Inc., Cary, NC). TL, TW, and age data were first transformed with natural logs (ln) to meet the assumptions of normality and homogeneity of variance. Tukey's honestly significant difference (HSD) test was used for post-hoc pairwise comparisons. Frequency distributions of size and age were compared by region with the Kolmogorov-Smirnov 2-sample (KS) test (Tate and Clelland, 1957). A chi-square (χ^2) test was used to determine whether sex ratios differed from a 1:1 ratio, overall and by region. Relationships of TW to TL were described first by fitting a linear regression to the ln-transformed variables and then by obtaining estimates (from the fitted regression coefficients) that corresponded to the parameters of the power function of $TW = aTL^b$. Analysis of covariance (ANCOVA) was used to compare the linearized slopes and intercepts among the regions.

Mean size at age (weighted by sample size) of the most common ages (3–7 years) was compared through the use of ANOVA with a Tukey's HSD test for post-hoc comparisons. Growth was modeled for observed TL and TW at age with von Bertalanffy growth equations, and tested for coincident curves with likelihood ratio tests (Kimura, 1980; Haddon, 2001) and Bonferroni's correction for multiple pairwise comparisons. Growth models were fitted with nonlinear regression by least squares in the following form:

$$TL_t = L_\infty(1-e^{-k(t)}), \quad (2)$$

$$TW_t = W_\infty(1-e^{-k(t)})^b, \quad (3)$$

where TL_t = TL at age t;
TW_t = TW at age t;
L_∞ = TL asymptote;
W_∞ = TW asymptote;
k = growth coefficient;
t = age in years; and
b = exponent derived from TW–TL regressions.

Table 1

Numbers of red snapper (*Lutjanus campechanus*) sampled in 2009 and 2010 for age and growth analysis from recreational catches in 6 regions of the Gulf of Mexico: South Texas, North Texas, Louisiana, Alabama, Northwest Florida, and Central Florida.

Region	Males	Females	Unknown sex	Total
South Texas	134	191	23	348
North Texas	111	93	20	224
Louisiana	131	132	5	268
Alabama	93	108	3	204
Northwest Florida	186	254	23	463
Central Florida	105	161	34	301
Total	761	938	108	1808

Models were forced through t_0=0 for comparison purposes because of a lack of small, young (<2 years old) individuals in all sample populations. For all statistical tests, significance was measured at an alpha level of 0.05.

Results

From 6 major recreational regions of the GOM, 1808 red snapper were sampled and a male-to-female ratio of 0.81:1.00 was calculated (Table 1). Among all regions, the majority of fish were small: mean TL of 540.19 mm (standard error [SE] 2.17) and mean TW of 2.40 kg (SE 0.04). On average, fish from Alabama were the largest fish, and the smallest fish were from the Florida regions (Table 2). Frequency distributions of TL and TW were significantly different among the regions; the largest proportion of small (<550 mm TL and <2.5 kg) fish were found in Northwest Florida (Fig. 2). No significant differences were noted between the sexes in the TL and TW frequency distributions (KS test: P=0.49 and P=0.65) and means (Tukey HSD test: P=0.68 and P=0.92).

Significant differences in TW–TL regression models were detected among the regions (ANCOVA test of

Table 2

Minimum, maximum, and mean values of (**A**) total length (TL), in millimeters, (**B**) total weight (TW), in kilograms, and (**C**) age, in years, of red snapper (*Lutjanus campechanus*) sampled from recreational catches in 6 regions of the Gulf of Mexico in 2009 and 2010. The 6 regions were South Texas, North Texas, Louisiana, Alabama, Northwest Florida, and Central Florida. Each section of this table is arranged in increasing order of mean values. Superscript letters indicate significant differences (<0.05) among the means. Within each row, means not connected with the same letter are significantly different (least-square means with Tukey's honestly significant different adjustment). Standard errors of the mean (SE) are provided in parentheses.

A	Northwest Florida	North Texas	Central Florida	South Texas	Louisiana	Alabama
n	*435*	*223*	*298*	*332*	*268*	*204*
Min TL	389	410	394	406	400	426
Max TL	880	900	780	722	821	880
Mean TL	497.15	525.94	530.43	552.10	560.81	604.19
(SE)	$(3.90)^a$	$(6.63)^b$	$(5.08)^b$	$(4.34)^c$	$(5.24)^c$	$(5.26)^d$
B	Northwest Florida	North Texas	Central Florida	Louisiana	South Texas	Alabama
n	*388*	*203*	*265*	*193*	*318*	*178*
Min TW	0.64	0.84	0.64	0.87	0.64	1.04
Max TW	9.16	10.25	7.52	8.71	9.22	12.7
Mean TW	2.10	2.18	2.21	2.45	2.54	3.28
(SE)	$(0.08)^a$	$(0.11)^a$	$(0.08)^a$	$(0.10)^b$	$(0.60)^b$	$(0.10)^c$
C	Central Florida	Northwest Florida	Louisiana	North Texas	Alabama	South Texas
n	*301*	*463*	*268*	*224*	*204*	*348*
Min Age	2	2	3	3	3	3
Max Age	10	9	21	33	16	13
Mean Age	4.06	4.17	4.72	4.78	4.79	4.86
(SE)	$(0.06)^a$	$(0.05)^a$	$(0.10)^b$	$(0.15)^b$	$(0.08)^b$	$(0.06)^b$

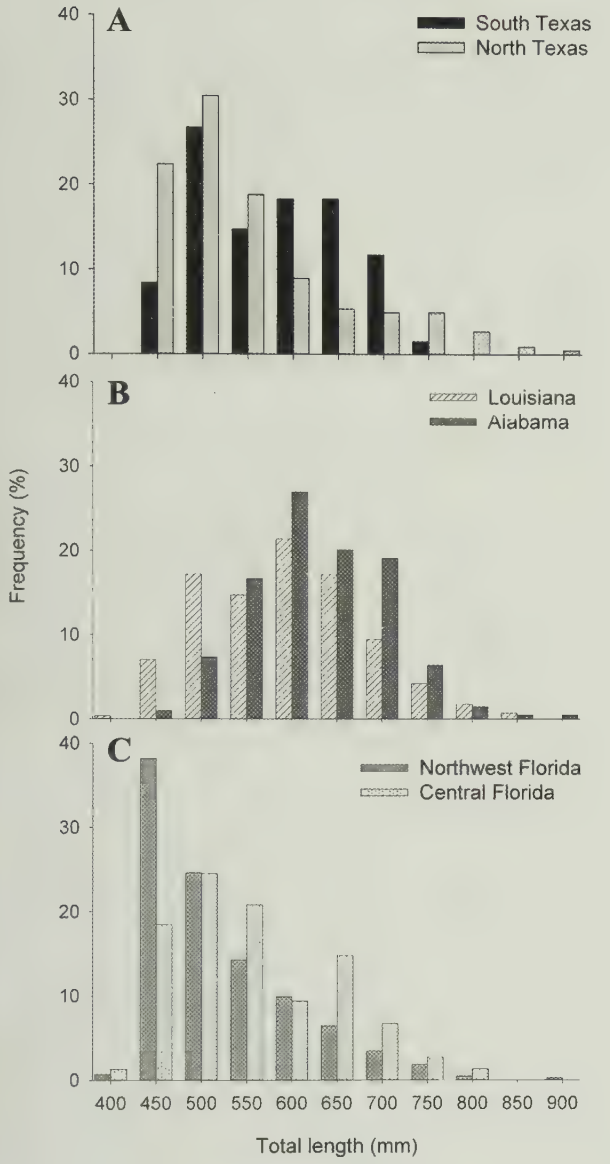

Figure 2

Frequency distributions of total length for red snapper (*Lutjanus campechanus*) sampled in 2009 and 2010 from 6 recreational fishing regions in the Gulf of Mexico: (**A**) South Texas (n=332) and North Texas (n=223), (**B**) Louisiana (n=268) and Alabama (n=204), and (**C**) Northwest Florida (n=435) and Central Florida (n=298).

homogeneity of slopes, $F_{5;1498}$=2.86; P=0.01; coefficient of determination [r^2]=0.92; ANCOVA test of equal intercepts, $F_{5;1498}$=2.95; P=0.01; r^2=0.92); therefore, separate models were fitted for each region (Table 3). No significant differences were observed between the TW–TL regressions for males and females (ANCOVA test of homogeneity of slopes, $F_{1;1504}$=0.11; P=0.89; r^2=0.91; ANCOVA test of equal intercepts, $F_{1;1504}$=0.13; P=0.87; r^2=0.91).

Ages were obtained from all 1808 transverse otolith sections. After the second reading, the readers reached agreement for 91.9% of the otoliths, with an APE of 1.08% (Table 4). Among all regions, red snapper were young (mean age: 4.51 years [0.03]) and 86% of individuals were between the ages of 3 and 5 years (Fig. 3). Fish from the 2 Florida regions had significantly smaller mean ages than fish from the other 4 regions (Table 2); these 2 regions also had the highest proportions of young fish (72% younger than 5 years) (Fig. 3). No significant differences were found between the sexes in the age distributions (KS test: P=0.77) and means (Tukey's HSD test: P=0.76).

Significant differences were observed among the regions in mean size at age for the most common ages of red snapper (Table 5). Fish from South Texas and Northwest Florida were consistently smaller in mean TL and TW at age than fish from the other regions (Fig. 4). Alabama fish were significantly larger at ages 4 and 5 than fish from the other regions. Fish from Louisiana and Central Florida were consistently larger than fish from both Texas regions and Northwest Florida and were not significantly different in mean TL at age (Table 5). Statistical comparisons of size at age for fish older than age 7 were not possible because of insufficient sample sizes.

All red snapper exhibited rapid growth until 6–8 years of age, after which growth slowed considerably (Fig. 5). Significant differences occurred among the von Bertalanffy growth models for the six regions (Table 6) but not between the sexes (likelihood ratio test df=2; TL model: χ^2 test=2.18, P=0.34; TW model: χ^2 test=4.18, P=0.12). All of the von Bertalanffy models differed from each other, except for the models of TL for Louisiana and Central Florida. Although significant differences were found among the growth models, pairwise comparisons of the model parameters were not reported because of the truncated range of the age data. The von Bertalanffy TL models for the North Texas and Alabama regions exhibited the largest L_∞, and the TL models for South Texas and Northwest Florida exhibited the smallest L_∞ and the largest growth coefficients (k) (Fig. 5A, Table 3). The von Bertalanffy growth models (both TL and TW) for North Texas, Louisiana, and Alabama produced similar growth coefficients (k≈0.2) (Fig. 5, A and B; Table 3).

Discussion

Among all of the regions, red snapper exhibited a truncated age structure with <1% of the sampled fish older than 10 years. A decade ago, Fischer et al. (2004) reported that 10% of red snapper examined from recreational catches of Texas, Louisiana, and Alabama were older than 6 years—a proportion that is more than double the occurrence of fish older than 6 years in our

Table 3

Total weight (TW)–total length (TL) regression models and TL and TW von Bertalanffy growth models for red snapper (*Lutjanus campechanus*) sampled in 2009 and 2010 from recreational catches in 6 regions of the Gulf of Mexico: South Texas, North Texas, Louisiana, Alabama, Northwest Florida, and Central Florida.

Region	TW–TL model	TL von Bertalanffy growth model	TW von Bertalanffy growth model
South Texas	$TW = 2.49\times10^{-8}(TL)^{2.90}$	$TL_t = 644.5(1-e^{(-0.4189(t))})$	$TW_t = 3.80(1-e^{(-0.4185(t))})^{2.90}$
North Texas	$TW = 7.85\times10^{-9}(TL)^{3.08}$	$TL_t = 908.2(1-e^{(-0.1905(t))})$	$TW_t = 10.57(1-e^{(-0.1953(t))})^{3.08}$
Louisiana	$TW = 1.66\times10^{-8}(TL)^{2.97}$	$TL_t = 771.0(1-e^{(-0.2988(t))})$	$TW_t = 7.69(1-e^{(-0.2537(t))})^{2.97}$
Alabama	$TW = 3.61\times10^{-8}(TL)^{2.85}$	$TL_t = 839.8(1-e^{(-0.2747(t))})$	$TW_t = 12.75(1-e^{(-0.2033(t))})^{2.85}$
Northwest Florida	$TW = 1.20\times10^{-8}(TL)^{3.02}$	$TL_t = 690.2(1-e^{(-0.3219(t))})$	$TW_t = 18.47(1-e^{(-0.1539(t))})^{3.02}$
Central Florida	$TW = 5.11\times10^{-9}(TL)^{3.15}$	$TL_t = 760.7(1-e^{(-0.3103(t))})$	$TW_t = 6.45(1-e^{(-0.3104(t))})^{3.15}$

study from those regions (5%). However, it should be noted that our study did not target larger or older fish from fishing tournaments as was done in the study described in Fischer et al. (2004). The oldest fish collected in our study (33 years) was approximately 20 years younger than the oldest specimens (54 and 57 years) in the GOM reported from previous studies (Wilson and Nieland, 2001; Mitchell et al., 2004; Allman and Fitzhugh, 2007).

The predominance of small, young fish may reflect the recent decline in size at age of red snapper, as well as the age truncation of the population due to overfishing (Berkeley et al., 2004; Allman and Fitzhugh, 2007; Nieland et al., 2007). The intense overfishing that occurred from the mid-1980s to early 1990s resulted in the most depleted state of the stock (Cowan et al., 2011; SEDAR[5]) and is a plausible reason why a scarcity of older fish was observed in our study (Allman and Fitzhugh, 2007; Nieland et al., 2007). Our findings are consistent also with recent stock assessments that indicate that fish older than 8 years are rarely caught in the GOM in the red snapper fisheries (SEDAR[1,5]).

Table 4

Differences between the 2 readers in average percent error (APE), coefficient of variation (CV), index of precision (D), percentages of agreement (O) for opaque annuli counts, and percentages of differences in age estimates (±1, 2, and 3 or more years) in red snapper (*Lutjanus campechanus*) otoliths after the first and second readings (n=1808). Red snapper were sampled in 2009 and 2010 from recreational catches in 6 regions of the Gulf of Mexico: South Texas, North Texas, Louisiana, Alabama, Northwest Florida, and Central Florida.

	1st reading	2nd reading
APE	1.77%	1.08%
CV	0.0177	0.0108
D	0.0125	0.0076
O	85.6%	91.9%
±1	13.54%	6.91%
±2	0.59%	1.00%
±3 or more	0.18%	0.18%

The size- and age-frequency distributions and growth models from this study indicate significant demographic differences in red snapper across the GOM. Small (≤550 mm TL), fast-growing fish dominated the recreational catches of South Texas and the eastern GOM. Larger (>600 mm TL), slower-growing fish constituted the majority of the catches in the northcentral and northwestern regions of the GOM, consistent with the findings of Fischer et al. (2004). Also, the larger representation of older fish and the more uniform distribution of age classes in the northern and western GOM are consistent with previous observations (Mitchell et al., 2004; Allman and Fitzhugh, 2007; SEDAR[7]). The dominant age classes (3–6 years) in our study appear to have been derived from the strong 2004, 2005, and 2006 year classes identified in the trawl surveys of the recent stock assessments (SEDAR[7]). These year classes may be linked to the recent decline in the GOM shrimp fisheries and corresponding reduction in bycatch of juvenile red snapper (Cowan, 2011; SEDAR[7]). The presence of these strong year classes among all 6 regions is similar to the consistent GOM-wide year-class patterns observed by Allman and Fitzhugh (2007). This combination of demographic differences and GOM-wide year-class consistency supports recent conclusions that red snapper form a metapopulation of semi-isolated assemblages that are demographically distinct but also highly influenced by mixing between assemblages (Gold and Saillant, 2007; Patterson, 2007; Saillant et al., 2010).

Our analyses of size at age and von Bertalanffy growth models indicate differences in the growth of red

[7] SEDAR (SouthEast Data, Assessment, and Review). 2009. Stock assessment of red snapper in the Gulf of Mexico: SEDAR update assessment, 224 p. [Available from http://www.sefsc.noaa.gov/sedar/.]

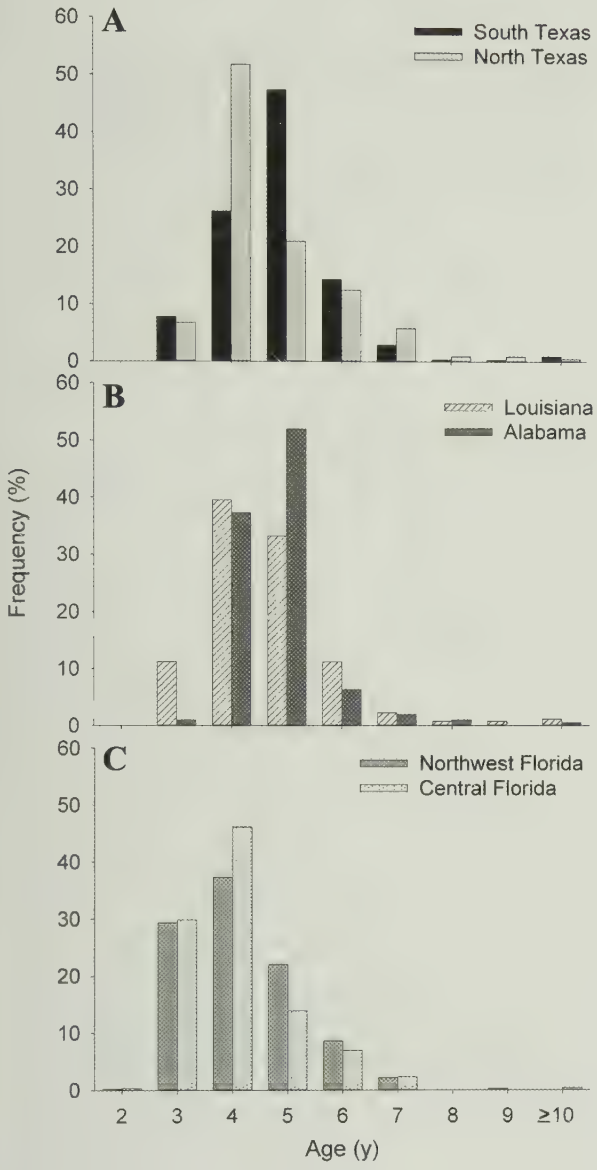

Figure 3

Frequency distributions of age (in years) for red snapper (*Lutjanus campechanus*) sampled in 2009 and 2010 from 6 recreational fishing regions in the Gulf of Mexico: (**A**) South Texas (n=348) and North Texas (n=224), (**B**) Louisiana (n=268) and Alabama (n=204), and (**C**) Northwest Florida (n=463) and Central Florida (n=301).

snapper across the GOM. Sample sizes were fairly consistent among the regions, allowing us to compare the trends in size at age and growth over the age ranges collected. The consistently smaller size at age, small estimates for maximum TL, and high k estimates for fish from South Texas and Northwest Florida are indicative of the highly truncated age structure and overexploitation in each region (Trippel, 1995; Rose et al., 2001; Berkeley et al., 2004; Nieland et al., 2007) and may be attributable also to variations in fishing regime (Fischer et al., 2004).

Although differences were found among the regional von Bertalanffy growth models, very few old fish were observed. As a result, the models were extrapolated beyond the range of data and may not be fully representative of each subpopulation. The absence of larger, older fish may strongly prevent the models from reaching an accurate asymptote (maximum size) (Haddon, 2001). Also, very few fish under the age of 3 years were included in our samples because of the minimum size limit on the recreational fishery (>406.4 mm maximum TL). Therefore, the models were forced through t_0=0 to more accurately predict juvenile growth, a selection that may increase k estimates. Nonetheless, the k estimates from this study were comparable to estimates from previous studies (Patterson et al., 2001b; Wilson and Nieland, 2001; Fischer et al., 2004).

In contrast to red snapper in South Texas, the slower-growing red snapper from North Texas appear to be more similar to fish in Louisiana and Alabama. These findings are consistent with reports of significant post-settlement movement of juveniles (0–2 years) between the northern and western GOM, as well as with otolith microchemistry analysis and larval transport studies that indicate that recruitment in the western GOM is subsidized by recruits from Louisiana (Patterson, 2007; Patterson et al., 2008; Johnson et al., 2009; Sluis, 2011). These observed differences may also be attributable to mixing of stocks between South Texas and Mexico because the Mexican stock is severely overfished and dominated by small, fast-growing fish (García et al., 2002). To date, no direct comparisons of red snapper age and growth from Mexican and U.S. waters have been made. However, in a recent otolith microchemistry study the source of recruits to the Texas continental shelf was determined to be derived from a combination of the Texas, Louisiana, and Mexico substocks (Sluis, 2011). Sluis (2011) also found a large contribution of the Louisiana substock to the eastern GOM. Therefore, the similarities observed in this study for red snapper between the Louisiana and Central Florida regions may be indicative of regional connectivity through offshore currents that flow clockwise along the outer continental shelf and potentially transport larvae and adults (Ohlmann and Niiler, 2005; Johnson et al., 2009).

The potential mechanisms for the observed demographic variation include environmental factors, fishing pressure (including localized population responses), habitat preference, and management regimes in the different regions (e.g., state regulations). Numerous environmental differences, including availability of suitable habitat, productivity of the surrounding ecosystem, and community structure could have contributed to the demographic dissimilarity among the regions. The productive, nutrient-rich waters of the Mississippi River plume have been shown to influence

Table 5

Analyses of variance (ANOVA) and Tukey's honestly significant difference test of mean total length at age (in millimeters) and total weight at age (in kilograms) for the most common ages (3–7 years) of red snapper (*Lutjanus campechanus*) sampled from recreational catches in 6 regions of the Gulf of Mexico in 2009 and 2010. The 6 regions were South Texas (STX), North Texas (NTX), Louisiana (LA), Alabama (AL), Northwest Florida (NFL), and Central Florida (CFL). Each section is arranged in increasing order of mean values. Letters are used to indicate significant differences (<0.05) among the means from the comparisons according to the Tukey's test. Within each row, means not connected with the same letter are significantly different.

	ANOVA				Comparisons according to Tukey's test					
Age	*n*	df	*F*	*P*	NFL	STX	NTX	CFL	LA	AL
Total length at age										
3	297	291	8.17	<0.0001	A	A	B	B	B	B
4	686	680	34.27	<0.0001	B	A	B	C	AC	C
5	536	530	18.59	<0.0001	AB	A	B	C	C	D
6	167	161	1.63	0.1557	–	–	–	–	–	–
7	49	43	4.74	0.0015	B	A	A	A	AB	A
Total weight at age										
3	238	232	8.58	<0.0001	A	B	AB	AB	A	AB
4	594	588	21.75	<0.0001	A	B	A	B	B	C
5	482	476	15	<0.0001	A	A	C	B	A	D
6	161	155	3.76	<0.0001	A	B	CD	D	AC	AC
7	47	41	0.9	0.4887	–	–	–	–	–	–

Table 6

Chi-square statistics (χ^2), degrees of freedom (df), and *P*-values for likelihood ratio tests used with Bonferroni's correction for multiple pairwise tests, for comparing von Bertalanffy growth models of total length (TL) and total weight (TW) among the 6 regions where red snapper (*Lutjanus campechanus*) were sampled in the Gulf of Mexico in 2009 and 2010. The regions were South Texas (STX), North Texas (NTX), Louisiana (LA), Alabama (AL), Northwest Florida (NFL), and Central Florida (CFL). Before regional models were compared, the full von Bertalanffy growth models for TL and TW (in which regions were fitted independently) were compared with the reduced models for TL and TW (by fitting all specimens).

	TL model			TW model		
Model comparison	χ^2	df	*P*	χ^2	df	*P*
Full–Reduced	291.1	2	<0.001	228.49	2	<0.001
NTX–STX	87.98	2	<0.001	94.78	2	<0.001
NTX–NFL	51.89	2	<0.001	22.83	2	<0.001
NTX–CFL	197.44	2	<0.001	92.74	2	<0.001
NTX–LA	129.31	2	<0.001	26.92	2	<0.001
NTX–AL	207.75	2	<0.001	89.77	2	<0.001
AL–STX	238.25	2	<0.001	183.09	2	<0.001
AL–NFL	343.75	2	<0.001	50.09	2	<0.001
AL–CFL	35.17	2	<0.001	13.12	2	0.001
AL–LA	54.97	2	<0.001	72.15	2	<0.001
LA–STX	82.62	2	<0.001	76.34	2	<0.001
LA–NFL	150.64	2	<0.001	20.25	2	<0.001
LA–CFL	0.94	2	0.624	30.49	2	<0.001
STX–NFL	58.26	2	<0.001	96.05	2	<0.001
STX–CFL	38.20	2	<0.001	46.96	2	<0.001
NFL–CFL	120.22	2	<0.001	78.22	2	<0.001

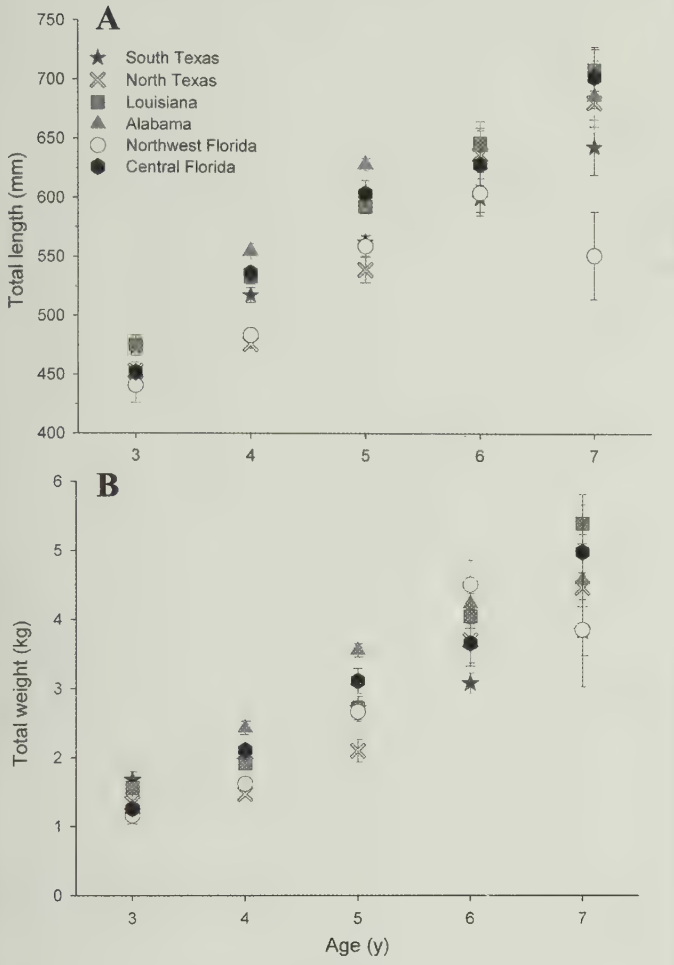

Figure 4

Mean (**A**) total length at age and (**B**) total weight at age of red snapper (*Lutjanus campechanus*) sampled in 2009 and 2010 from 6 recreational fishing regions in the Gulf of Mexico: South Texas, North Texas, Louisiana, Alabama, Northwest Florida, and Central Florida. Error bars represent ±1 standard error of the mean.

fishery production through increased growth rates of fish in the northcentral GOM when compared with other regions (DeVries et al., 1990; Grimes, 2001) and may be more conducive to growth in the Louisiana and Alabama regions (Fischer et al., 2004). Because habitat complexity and patchiness vary greatly throughout the GOM, the amount and suitability of preferred habitat, as well as prey availability, quantity, and quality, may affect the observed differences in age and growth.

Age-specific habitat preference also may play a role in the differences observed in our study. Red snapper undergo an ontogenetic shift in habitat during their first several years of life, settling on a variety of low-relief habitats and then moving to higher relief habitats with greater complexity, usually into deeper waters (>30 m) farther offshore (Workman et al., 2002; Geary et al., 2007; Wells et al., 2008;). Previous studies have hypothesized that older red snapper (>6–8 years) in northwestern GOM become less associated with reefs once they reach a predation size threshold that allows them to emigrate away from both natural and artificial structures to alternative habitats with lower relief (Render, 1995; Nieland and Wilson, 2003; Mitchell et al., 2004). Typically, recreational fishermen (in head boats and charter boats in particular) are limited by trip time and bag size; therefore, they fish relatively close to shore and presumably at shallower depths (<40 m), and, as a result, their catches are dominated by younger (<4 years) age classes (Allman and Fitzhugh, 2007; SEDAR[7]).

It is important to note also that red snapper have never been distributed uniformly across the GOM (Porch et al., 2007; SEDAR[1]). Data for commercial landings over the past century indicate a recent shift in the center of abundance from the northeastern (Alabama and Florida regions) to the northwestern GOM (Louisiana region) (Porch et al., 2007). The distribution of fishing sectors has also shifted with the center of abundance and varies significantly across the GOM. The commercial red snapper fishery and bycatch from the shrimp fishery constitute the main sources of fishing mortality in the western GOM, and the recreational fishery accounts for the greatest source of fishing mortality in the eastern GOM (GMFMC[2]). In addition, variations in fishing regimes (i.e., vessel type, trip length, distance from shore, and depth fished) within the recreational fishery may influence the size and age of the fish that are caught in each region (Fischer et al., 2004). Therefore, the uneven distribution of the fishing sectors, combined with their differing management plans (quotas, size limits, and trip and bag limits) and fishing regimes, may also significantly influence the formation of demographic stocks of red snapper in the GOM.

Several compensatory responses to fishing pressure, including faster growth and early maturation, have been noted in the stock of red snapper in the GOM (Jackson et al., 2007; Nieland et al., 2007; Allman et al.[8]; Kulaw, 2012;) and were observed in our study. It appears that red snapper devote more of their energy as younger fish to reproduction: fish sampled in a companion study showed that 75% maturity is occurring by age 3 across the GOM (Kulaw, 2012), whereas

[8] Allman, R. J., B. K. Barnett, N. Evou, R. A. Farsky, J. Keesee, and P. Carlson. 2009. Red snapper (*Lutjanus campechanus*) otolith aging summary 2003 to 2008. NMFS Panama City Laboratory Contribution Series 09–15, 10 p. [Available from http://www.sefsc.noaa.gov/sedar/.]

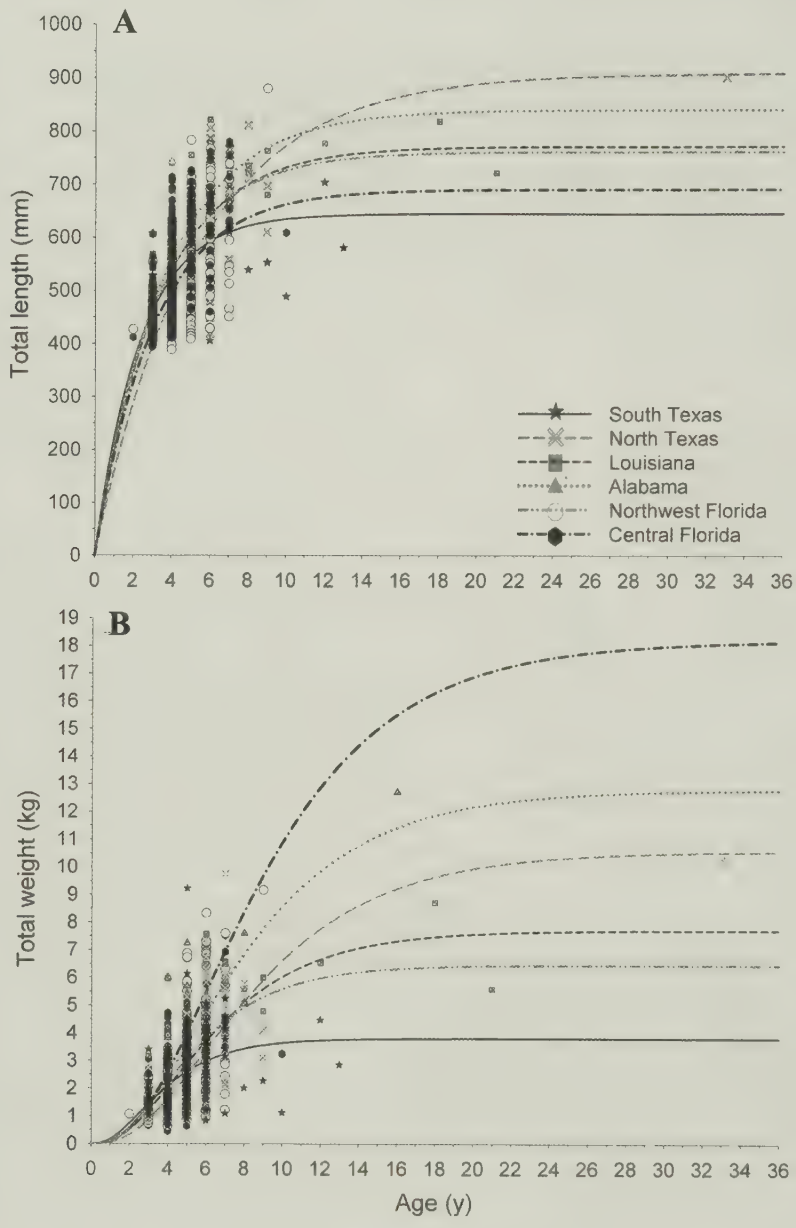

Figure 5

Observed (**A**) total length at age and (**B**) total weight at age for red snapper (*Lutjanus campechanus*) sampled in 2009 and 2010 from 6 recreational fishing regions in the Gulf of Mexico: South Texas, North Texas, Louisiana, Alabama, Northwest Florida, and Central Florida. Plotted lines represent the region-specific von Bertalanffy growth functions fitted to the data.

fish sampled in previous in previous studies showed that 75% maturity was reached between 4 and 8 years of age (Woods et al., 2003; Fitzhugh et al.[9]; Jackson et al., 2007). Truncating the age distribution of the stock decreases its reproductive potential and limits the rate of population recovery because fecundity increases with size and age and longevity extends reproduc-

[9] Fitzhugh, G. R., M. S. Duncan, L. A. Collins, W. T. Walling, and D. W. Oliver. 2004. Characterization of red snapper (Lutjanus campechanus) reproduction: for the 2004 Gulf of Mexico SEDAR. NMFS Southeast Fisheries Science Center Panama City Laboratory Contribution Series 04-01, 29 p. [Available from http://www.sefsc.noaa.gov/sedar/.]

tion across a long period of time (Trippel et al., 1997; Berkeley et al., 2004; Palumbi, 2004; Kulaw, 2012).

Conclusions

This study documented the highly truncated age structure of the recreational catches of red snapper and highlighted the demographic differences in size, age, and growth of this species across the GOM. The potential mechanisms in the observed demographic variation include environmental differences, fishing pressure, habitat preference, and management regimes; however, no definitive conclusion about cause and effect can be made. Implications of these differences along with the theory that red snapper form a metapopulation in the GOM should be considered in future stock assessments and management decisions.

The most recent stock assessment indicates that red snapper in the western GOM are beginning to recover from overfishing (SEDAR[5]). However, we documented on the basis of recreational catches across the GOM that red snapper continue to exhibit a severely truncated age structure. It is expected that as the stock rebuilds, there will be a shift to an older age structure (Allman and Fitzhugh, 2007; SEDAR[5]). An increase in red snapper biomass has been observed in the fisheries; however, an age shift was not readily apparent in our study, the latest stock assessments, or other recent studies (Allman and Fitzhugh, 2007; SEDAR[5]). Identification and protection of the strong year classes of 2004–06 will allow for stock recovery and help eliminate the truncated age structure as more fish reach maximum spawning potential (Berkeley et al., 2004; Palumbi, 2004).

Acknowledgments

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A validated, minimally deleterious method for aging sturgeon

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Abstract—To determine the most suitable aging structure for sturgeons, band counts of transverse sections of otoliths and the pectoral-, dorsal-, pelvic-, and anal-fin rays of Gulf sturgeon (*Acipenser oxyrinchus desotoi*) were compared. The otoliths and dorsal-, pelvic- and anal-fin rays produced inconsistent band patterns, but bands formed in the first (fin spine) and second marginal pectoral-fin rays were easily read and the most consistent. The index of average percent error and the coefficient of variation between final ages from the fin spines and the second marginal fin ray were 0.75% and 1.06%, respectfully. Percent agreement between the assigned age of the fin spine and second marginal fin ray was high. The fin spine is commonly used to age sturgeon species; however, removal of the fin spine was considered potentially deleterious, especially to larger sturgeon. Sampling the second marginal fin ray appeared to be less harmful. Annual band formation in the second marginal fin ray for 3 Gulf sturgeon was validated with the use of oxytetracycline. We propose the second marginal pectoral-fin ray as an acceptable and less harmful alternative structure to the fin spine for aging Gulf sturgeon and recommend its use for other threatened and endangered sturgeon species. An extraction method of minimal invasiveness is presented here for field removal of the second marginal pectoral-fin ray from live sturgeon.

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Many sturgeon species are prized for their meat and caviar, and heavy fishing effort during the 19th century greatly reduced population sizes around the world (Birstein, 1993). Of the 26 sturgeon species assessed by the International Union for Conservation of Nature and Natural Resources and listed on the IUCN Red List of Threatened Species, 18 (69%) are listed as endangered or critically endangered (vers. 2012.2, http://www.iucnredlist.org, accessed March 2013). Additionally, 20 of these species are listed as decreasing in population: only 4 sturgeon species are listed as increasing or stable and 2 have unknown population trends. North America is home to 9 species of sturgeon, 8 of which are listed as threatened or endangered under the U.S. Endangered Species Act (U.S. Fish and Wildlife Service, Species Reports, Environmental Conservation Online System, http://ecos.fws.gov/tess_public, accessed March 2013; NMFS[1]): shortnose (*Acipenser brevirostrum*), green (*A. medirostris*), Atlantic (*A. oxyrinchus*), Gulf (*A. oxyrinchus desotoi*), white (*A. transmontanus*), pallid (*Scaphirhynchus albus*), shovelnose (*S. platorynchus*), and Alabama (*S. suttkusi*). Sturgeon generally exhibit slow growth and late maturation—life history characteristics that make populations slow to recover from exploitation. Moreover, these life history traits, as well as habitat issues, equate to a difficult recovery process.

Age estimates of several species of sturgeon in North America have been evaluated by removing and observing the banding patterns of calcified structures, such as the first marginal pectoral-fin ray, otoliths, scutes, opercula, and sphenoids (Brennan and Cailliet, 1989; Nakamoto et al.[2]; Rossiter et al., 1995; Stevenson and Secor, 1999; Hurley et al., 2004; Jackson et al., 2007; Bruch et al., 2009). Although it is not a true spine, the first marginal pectoral-fin ray of sturgeons commonly is called the *fin spine*, and hereafter it will be referred to as such in this article. All structures except the fin spine have proved to be detrimental to or have required sacrifice of the fish (Brennan and Cailliet, 1989); therefore, the most widely used aging structure for sturgeon species is the fin

[1] NMFS (National Marine Fisheries Service). 2013. Endangered and threatened marine and ana–dromous fish. [Available from http://www.nmfs.noaa.gov/pr/species/esa/fish.htm, accessed March 2013.].

[2] Nakamoto, R. J., T. T. Kisanuki, and G. H. Goldsmith. 1995. Age and growth of Klamath River green sturgeon (Acipenser medirostris). U.S. Fish and Wildlife Service Project # 93-FP-13, 20 p. [Available from Yreka Fish and Wildlife Office, U.S. Fish and Wildlife Serice, 1829 South Oregon St., Yreka, CA 96097.]

Figure 1

The approximate range (the shaded area) and major spawning rivers of Gulf sturgeon (*Acipenser oxyrinchus desotoi*) in the Gulf of Mexico. Structures for aging Gulf sturgeon were collected from sturgeon captured in the Choctawhatchee River in 2010, from archived specimens from the Apalachicola and Choctwahatchee rivers in 2010, and from an archive of Gulf sturgeon captured on the Suwannee River in 1980–90.

spine (Rien and Beamesderfer, 1994; Rossiter et al., 1995; Stevenson and Secor, 1999; Berg, 2004; Hurley et al., 2004; Whiteman et al., 2004; Allen et al., 2009). Kahn and Mohead (2010) advised against the use of the fin spine in mature shortnose, Atlantic, Gulf, and green sturgeon because of increased reader error, abnormal regrowth, and an increased chance of deleterious effects. To reduce harm to endangered and threatened species and to increase standardization for aging protocols, options other than the fin spine should be developed.

Another important aspect of age and growth that is often ignored is the validation of annual band formation in aging structures (Beamish and McFarlane, 1983; Campana, 2001). Despite the widespread use of the pectoral spine for age estimation (Rien and Beamesderfer, 1994; Rossiter et al., 1995; Morrow et al., 1998; Stevenson and Secor, 1999; Hurley et al., 2004; Whiteman et al., 2004), successful validation of annual band formation in the pectoral spine has been published for only 2 North American sturgeon species: white (Rien and Beamesderfer, 1994) and lake (Rossiter et al., 1995) sturgeon. Accurate age estimation is critical because it forms the basis for calculating growth and mortality rates, age at maturity, and estimates of longevity, all of which are essential for the production of accurate population models.

For this study, we had the following objectives: 1) to evaluate and compare banding patterns from the pectoral spines, fin rays, and otoliths of Gulf sturgeon; 2) to determine which aging structure, for which removal is nonlethal and minimally deleterious, provides the most accurate and consistent band counts; 3) to validate the frequency of band formation within the preferred aging structure; and 4) to provide the field and laboratory methods to remove, process, and interpret the validated aging structure for Gulf sturgeon.

Materials and methods

Sampling protocol

To determine the best aging structure for Gulf sturgeon, 14 frozen Gulf sturgeon from previous mortality events on the Choctawhatchee and Apalachicola rivers (Fig. 1) were sampled in 2010. The fin spine, second marginal pectoral-fin ray (the fin ray just posterior to the fin spine), pectoral-, dorsal-, pelvic-, and anal-fin rays, and sagittal otoliths were removed, cleaned, and

sectioned to determine the most suitable aging structure for Gulf sturgeon. Additionally, an archive of air-dried fin spines from Gulf sturgeon collected on the Suwannee River in 1980–90 was donated for inclusion in future age and growth analysis (K. Sulak, U.S. Geological Survey). Fin spines from sturgeon collected in the Pascagoula River likewise were processed for future studies (M. Peterson, Univ. Southern Mississippi). In many cases, the second marginal pectoral-fin ray was sampled along with the fin spine: these samples were used to compare band counts between these structures.

Processing of structures

Fin spines, fin rays, and otoliths The entire left pectoral, left pelvic, dorsal, and anal fins were removed at the point of articulation from each of the 14 archived Gulf sturgeon. With a scalpel, the fin spine and approximately 5 leading marginal rays were isolated from each fin. Excess tissue was removed from between each hemitrich of the fin rays with a stiff brush and forceps. In extreme cases, samples were boiled (2–5 min) to ease cleaning. Air-dried fin rays and small, fragile spines (<5 mm in diameter) were mounted in Loctite 0151 Hysol[3] epoxy (Henkel Corp., Rocky Hill, CT) and allowed to harden for 24 hours. Large fin spines (>5 mm in diameter) were not mounted in epoxy because of their rigid nature; to simplify mounting and obtain the best sections, the articulating process of the fin spine was removed by using a Hillquist Thin Section Machine (Hillquist Inc., Denver).

Through the use of an IsoMet low speed saw (Buehler, Lake Bluff, IL) with a Norton grinding wheel (Saint-Gobain, Courbevoie, France), a minimum of 3 transverse sections approximately 0.3–0.6 mm thick were made starting at the proximal end of the fin spine or ray. Sections were mounted on microscope slides with the clear mounting medium Cytoseal (Thomas Scientific, Swedesboro, NJ) and examined under a dissecting microscope at magnifications of 10–75× that varied depending on the size of the section. Transmitted or reflected light was used on the basis of which one best elucidated band formations. Digital images of each section were taken with a MicroPublisher 5.0 real-time viewing camera (QImaging, Surrey, Canada) mounted on an RZ Series CMO stereo microscope (Meiji Techno America, Santa Clara, CA). Growth bands were counted by using standard aging methods (Brennan and Cailliet, 1989; Morrow et al., 1998; Stevenson and Secor, 1999), whereby a band pair consisted of one opaque and one translucent ring that was continuous around the section.

Both sagittal otoliths were removed from the 14 archived Gulf sturgeon, cleaned in fresh water, and allowed to air dry. One otolith from each sturgeon was mounted in 2 Ton Epoxy (ITW Devcon, Danvers, MA) and sectioned according to Bruch et al (2009). Sections (0.5 mm) were mounted on a microscope slide with Cytoseal mounting medium. Digital images were taken of each otolith section according to the method described previously for fin rays. The second whole otolith was stored dry and archived.

Each structure was evaluated qualitatively on the basis of 3 criteria: 1) invasiveness of the removal; 2) practicality of sampling in the field; and 3) consistency and clarity of the banding pattern. The structure for which removal was the least harmful, while also providing consistent banding patterns, was considered the "best" aging structure for Gulf sturgeon.

Precision and bias To measure reproducibility and test whether the second marginal pectoral-fin ray was a comparable aging structure to the commonly used fin spine, average percent error (APE) (Beamish and Fournier, 1981) between band counts for each structure was calculated when both were available:

$$APE_j = 100\% \times \frac{1}{R} \sum_{i=1}^{R} \frac{|X_{ij} - X_j|}{X_j}, \quad (1)$$

where X_{ij} = the ith age determination of the jth fish;
X_j = the mean age of the jth fish; and
R = the number of times each fish was aged.

Age estimates between the fin ray and fin spine were measured for precision with the coefficient of variation (CV) (Chang, 1982):

$$CV_j = 100\% \times \frac{\sqrt{\sum_{i=1}^{R} \frac{\left[X_{ij} - X_j\right]^2}{X_j}}}{X_j}, \quad (2)$$

where CV_j = the age precision estimate for the jth fish.

Average percent error was calculated for each fish for which samples of both a fin spine and a second marginal fin ray existed, and the results were presented as an index. Similarly, CV was averaged across all samples to produce a mean value. Percent agreement (PA) of age estimates from fin rays and fin spines was also calculated. Gulf sturgeon without a fully formed band pair (band count=0) were not included in analyses because their inclusion artificially inflated the APE, and CV could not be calculated for those fish. Precision and bias of band counts for each structure was examined by using a 1:1 bias plot.

Field proofing After the best aging structure had been determined, a protocol was developed for removal of fin rays from live sturgeon. Fin rays were collected opportunistically from Gulf sturgeon during ongoing collaborative gillnet surveys conducted by researchers from Delaware State University, the University of Southern

[3] Mention of trade names or commercial companies is for identification purposes only and does not imply endorsement by the National Marine Fisheries Service, NOAA.

Mississippi, and the U.S. Fish and Wildlife Service. Fork length (FL), total length (TL), and weight of each sturgeon were recorded in centimeters and kilograms, respectively, as part of these surveys. Collection of fin rays primarily occurred in the fall months, and samples were collected on the Yellow, Choctawhatchee, and Apalachicola rivers from September 2010 through early November 2012.

Validation of aging structure As part of the standardized sampling practices used for ongoing collaborative surveys, approximately 100 Gulf sturgeon received a dose of 10 mg of oxytetracycline (OTC) per kilogram of body weight, which was injected intramuscularly (Baremore and Rosati, 2011). An aging structure from each recaptured, OTC-injected Gulf sturgeon was removed and sectioned according to the methods described previously. These sections were then viewed under a dissecting microscope with reflected UV light to illuminate any potential chemical mark that the injected OTC had incorporated within the structure. The placement of the chemical mark was evaluated in comparison with subsequently formed band pairs and with time at large to determine frequency of band formation.

Results

Sampling protocol

Fin spines Removal of fin spines was considered to be invasive and possibly harmful to the fish because of bleeding, potential for the incised spine to snag in debris, and loss of hydrodynamics while swimming. The practicality of removal in the field was somewhat limited, especially for Gulf sturgeon >120 cm FL, because a large hack saw was generally required. The banding pattern was consistent until about 15 band pairs were counted, a threshold after which band pairs often were compressed and reading bands became difficult (Fig. 2A). In many cases, the second marginal fin ray was incorporated in the spine, an occurrence that could obscure bands and make band counts less reliable. Because of their historic use and mostly clear banding patterns, fin spines were not excluded as an aging structure; however, they were considered not ideal for aging live sturgeon.

Fin rays: dorsal, pelvic, and anal Removal of dorsal-, pelvic-, and anal-fin rays from Gulf sturgeon was minimally invasive; these fin rays were small and not rigidly structured and could be removed easily. The required tools for removal were a scalpel, forceps, and wire cutters. Once sectioned, banding patterns were visible; however, bands were indistinct and inconsistent in relation to the other structures (for image of dorsal-fin rays, see Fig. 2B). Although easy to remove and not harmful to the sturgeon, these fin rays were excluded as aging structures because of the lack of clarity of the bands.

Otoliths Otolith removal was maximally invasive because of their location in the head and could only be performed on mortalities. Otoliths were also very difficult to retrieve because of the bony structure and size of Gulf sturgeon. Band pairs could be difficult to distinguish and were inconsistent among individuals; therefore, band counts could not be quantified for this structure (Fig. 2C). The use of otoliths for aging Gulf sturgeon was determined to be neither feasible nor acceptable for a threatened species.

Second marginal pectoral-fin ray The second marginal pectoral-fin ray was larger and more rigidly structured than the fin rays from other fins of Gulf sturgeon. Although removal of this fin ray was slightly more invasive because of its size, the effect of its removal on the sturgeon was considered to be negligible. The removal of this fin ray was relatively easy in the field and required the same tools as the other fin rays. Furthermore, band formation was mostly clear and consistent for the majority of samples (Fig. 2D). Therefore, on the basis of our established criteria, the second marginal pectoral-fin ray was chosen as the best structure for aging Gulf sturgeon.

Removal of the second marginal fin ray was simple and could be completed by an experienced biologist in less than 1 min. The tools necessary for removal were small, inexpensive, and portable—notable differences in comparison with the hacksaw that was often required for the removal of fin spines. The second marginal fin rays of Gulf sturgeon >150 cm FL were more difficult to remove and often required a larger initial incision because of the diameter of the fin ray. The edges were often fractured from the force required to excise the sample, but the fin ray generally could be salvaged if the removed segment was at least 2 cm in length.

Captured Gulf sturgeon were placed in a holding tank with their ventral surface facing up. A size 10 scalpel was used to make a parallel cut on each side of the second marginal pectoral-fin ray approximately 2.5 cm away from the point of articulation. This process acted to separate the second marginal fin ray from the spine and third marginal fin ray while allowing us to avoid major blood vessels. Wire cutters were then used to free the segment (1.0–1.5 cm) by snipping each end of the parallel cut. The fin ray segment was removed with forceps, and a mixture of Betadine antiseptic solution (Purdue Products, Stamford, CT) and petroleum jelly was placed over the site for prophylactic purposes. Sampled fin rays were placed in labeled paper or plastic bags and allowed to air dry before processing. Several Gulf sturgeon that were sampled for age analysis were recaptured up to 6 months after the initial capture to assess healing of the fin.

Bleeding at the site of the incision was common but was generally minimal and stopped within 5 min of the cessation of the procedure. Recaptured individuals were healed completely 6 months after fin removal, and the fin showed less irritation at the removal site than

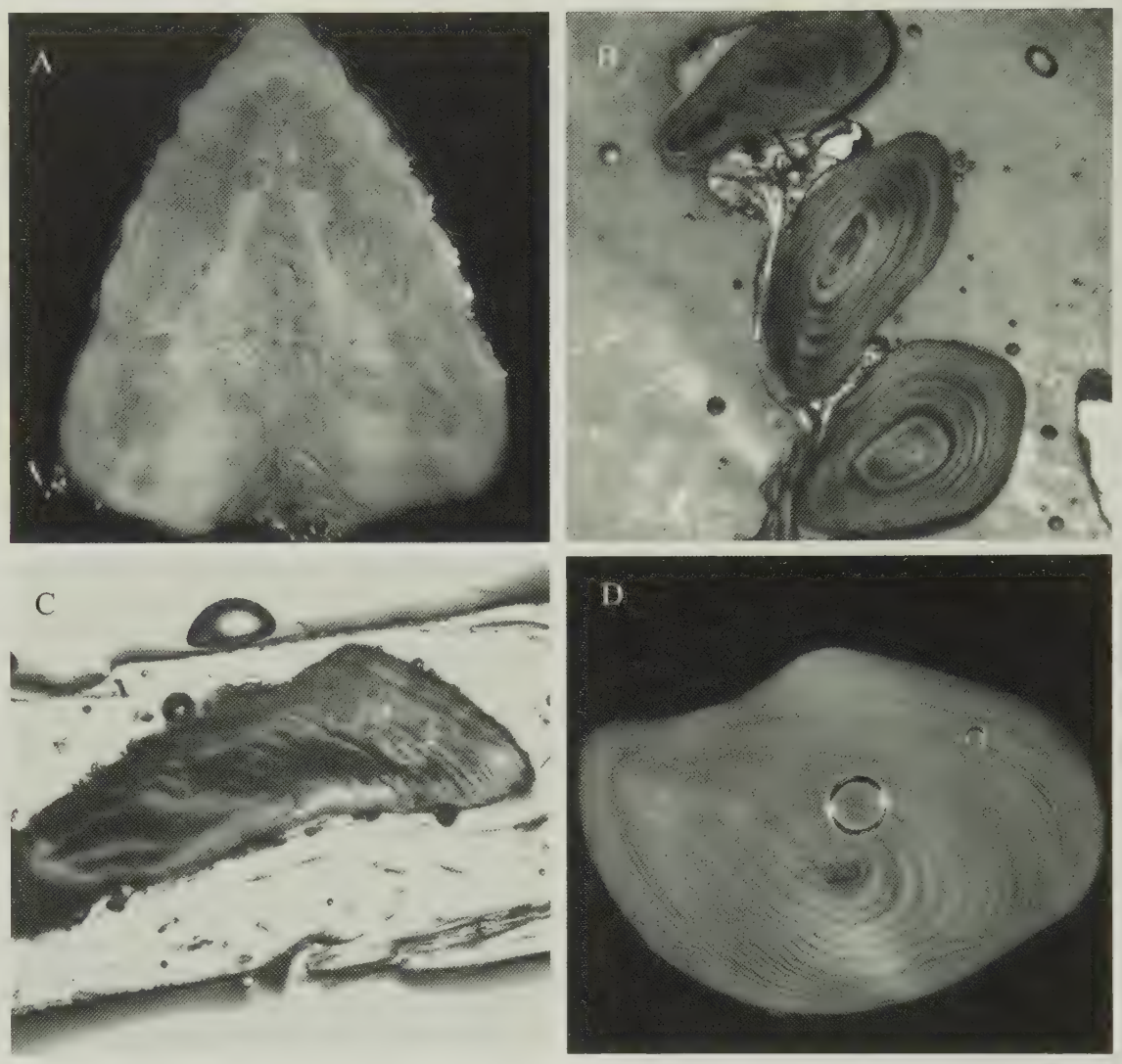

Figure 2

Examples of sections of Gulf sturgeon (*Acipenser oxyrinchus desotoi*) used in this study for comparison of age structures: (**A**) fin spine with incorporated fin rays, (**B**) dorsal-, pelvic-, and anal-fin rays, (**C**) otolith, and (**D**) second marginal pectoral-fin ray. Fin spines and fin rays were sectioned at 0.3–0.6 mm and otoliths were sectioned at 0.5 mm with an IsoMet low speed saw. Fin spines and second marginal pectoral fin-rays provided the clearest banding patterns. Fin rays from the dorsal, pelvic, and anal fins were inconsistent and indistinct, and sectioned otoliths provided unreliable banding patterns. Structures for aging Gulf sturgeon were collected from sturgeon captured in the Choctawhatchee River in 2010, from archived specimens from the Apalachicola and Choctwahatchee rivers in 2010, and from an archive of Gulf sturgeon captured on the Suwannee River in 1980–90.

did those fins with commonly applied external tags (Fig. 3, A and B). Removal sites were healed dramatically in comparison with the sites where fin spines had been sampled (Fig. 3C).

Overall, the second marginal fin ray produced consistent, clear, and reliable banding patterns. Band count comparisons between the second marginal fin ray and fin spine showed little systematic bias between the structures for counts of up to 25 bands (Fig. 4). Precision and PA estimates were high for band counts between the fin spine and second marginal fin ray: n=222; CV=0.75%; APE=1.06%; PA=96.46% (±0 band pairs), 98.23% (±1 band pair), and 100.00% (±2 band pairs). Therefore, the second marginal fin ray was considered a robust aging structure and appears to be a valid substitute for the fin spine for aging analysis.

Figure 3

Ventral surface of Gulf sturgeon (*Acipenser oxyrinchus desotoi*) pectoral fins (**A**) just after removal of a segment of the second marginal pectoral-fin ray, (**B**) 6 months after removal of that segment, and (**C**) immediately after removal of the right fin spine and left second marginal fin ray. An arrow in each panel indicates the place where the second marginal fin ray was removed, and the star in the final panel marks the site of the fin spine removal. Second marginal pectoral-fin rays were removed from live Gulf sturgeon in the Choctawhatchee and Apalachicola rivers during 2010, and these photos indicate that removal of this fin ray is less invasive than removal of fin spines.

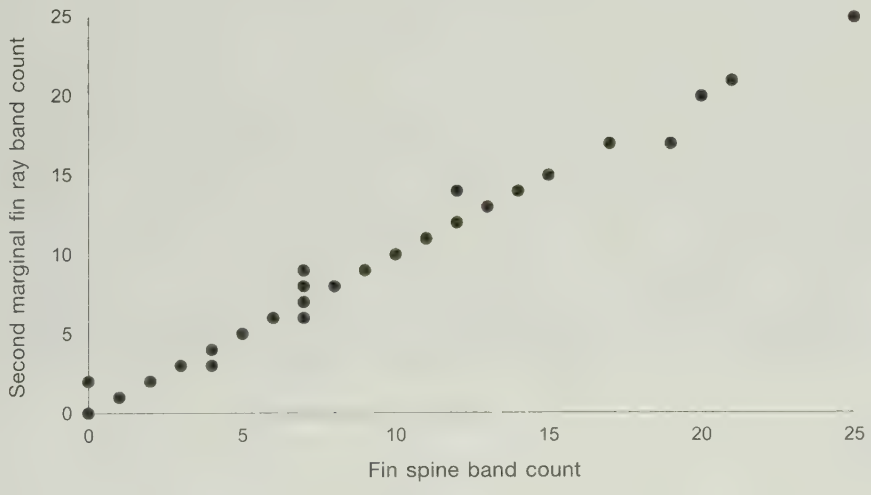

Figure 4

Final band counts of pectoral fin spines and second marginal pectoral-fin rays from Gulf sturgeon (*Acipenser oxyrinchus desotoi*) (n=222) captured in the Choctawhatchee River in 2010, from an archive of specimens that were collected in the Apalachicola and Choctwahatchee rivers in 2010, and from an archive of Gulf sturgeon captured on the Suwannee River in 1980–90. The graph indicates little systematic difference up to 25 band pairs between band counts from the second marginal fin ray and band counts from the fin spine.

Validation of aging structure

Annual band formation was validated for 3 Gulf sturgeon that were recaptured one year after being marked with OTC and for 1 Gulf sturgeon that was recaptured after 6 months. Visual assessment of the sections showed that, for sturgeon with 6, 7, and 8 total band pairs, 1 band pair was fully formed after the chemical mark from the OTC injection (Fig. 5). A Gulf sturgeon recaptured in October after being injected in Febru-

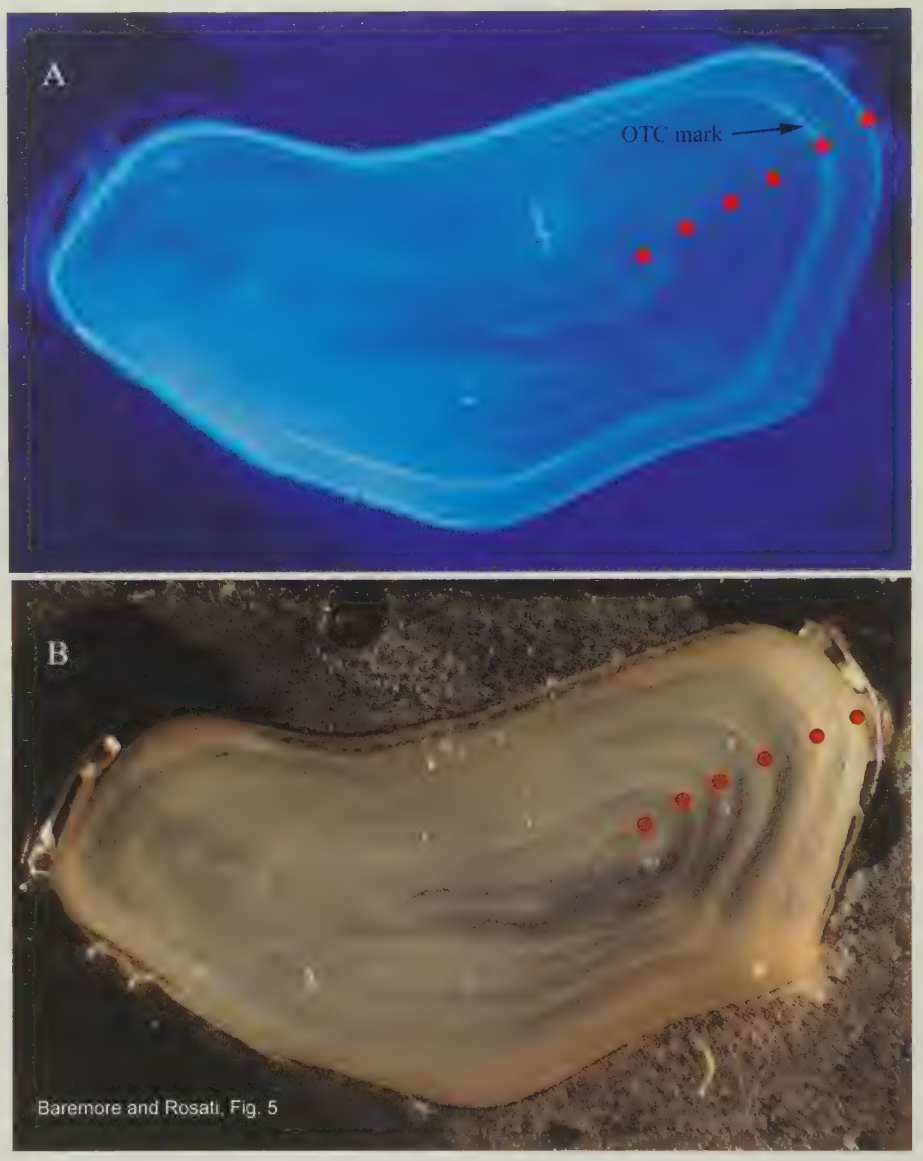

Figure 5

Sectioned second marginal pectoral-fin ray of a Gulf sturgeon (*Acipenser oxyrinchus desotoi*) (**A**) under UV light and (**B**) under natural light. Both images show the same sturgeon one year after it was captured and marked chemically with oxytetracycline (OTC). The fluoresced mark, indicated by the arrow, shows the marking incorporated on the initial capture date of 8 October 2009. Red dots indicate band pairs and show that, by the recapture date of 11 October 2010, one complete band pair had formed after the chemical mark. Both capture events occurred in the Choctawhatchee River.

ary of that year had 15 band pairs; a partially formed, translucent band was evident after the chemical mark. However, fin rays from 8 other recaptured sturgeon did not fluoresce under UV light. This outcome indicates that OTC was not incorporated into the bony structures of many of the Gulf sturgeon that were injected with it and that doses may have been too low for validation purposes.

Discussion

The second marginal pectoral-fin ray is not an unknown structure for age and growth studies, although its use as a primary aging structure has not been discussed previously. Brennan and Cailliet (1989) noted that bands from this fin ray were consistent with the bands from fin spines but did not evalu-

ate the use of this fin ray as an aging structure. We found that the second marginal fin ray was simple to remove and that sections provided reliable and clear band counts.

Otoliths and fin spines are commonly used to age fishes—fin spines being the most prevalent for sturgeon species (Huff[4]; Brennan and Cailliet, 1991; Rossiter et al., 1995; Stevenson and Secor, 1999; Everett et al., 2003). Although otoliths often are considered the best structure for aging fish (Casselman, 1987; Campana, 2001), band formation in otoliths of Gulf sturgeon was unreliable and difficult to interpret. Similar difficulties have been noted for other sturgeon species (Brennan and Cailliet, 1989); however, bomb radiocarbon dating has been used to validate band formation for 46 otoliths of lake sturgeon (Bruch et al., 2009). Fin spines provide mostly clear and interpretable banding patterns, and their use has been validated for several species (Rien and Beamesderfer, 1994; Rossiter et al., 1995), but their removal has been discouraged for threatened and endangered sturgeon species because of the possibility of deleterious effects (Kahn and Mohead, 2010).

Although our precision and PA estimates were very high for band counts between the fin spine and second marginal fin ray, our age assignments could have been somewhat biased by the methods used. Because the fin spine and second marginal fin ray were often on the same slide, band counts were made in conjunction with both structures, rather than independent of each structure. Future comparative studies should randomize the order in which fin rays and spines are read.

Of the Gulf sturgeon that previously had been injected with OTC, 12 were recaptured and sampled over the course of this study. However, formation of annual bands in the second marginal fin ray was validated for only 3 of those Gulf sturgeon. Ages could not be validated for the other recaptured Gulf sturgeons because OTC injections failed to produce a chemical mark in 8 of those fish. This low rate of OTC incorporation may have been due to the methods used: Rien and Beamesderfer (1994) reported a 98% detection rate of OTC chemical marks in the fin spines of white sturgeon when they used a concentration of 25 mg/kg and Rossiter et al. (1995) used a dose of 35 mg/kg to validate band formation in lake sturgeon . The use of a dose of 10 mg/kg in our study likely led to the high failure rate, and future validation studies for sturgeon species should use at least 25 mg/kg. Loss of OTC at the injection site was also noted; therefore, a slow injection followed by pressure applied to the site is recommended as well. Despite the low detection rate, we feel confident that band pairs in the second marginal fin rays are annual marks.

Although the set of methods used in this study was developed for Gulf sturgeon, it likely is widely applicable to other sturgeon species worldwide. Researchers have advised that caution be taken when the fin spine is used to age sturgeon because of difficulty with identification of growth bands (Hurley et al., 2004; Jackson et al., 2007) and because of the potentially harmful effects of removing the fin spine from large individuals (Kahn and Morehead, 2010). Additionally, the use of the second marginal fin ray may also be appropriate for species related to sturgeon, such as paddlefish. Accurate life history information is crucial for the development of population assessments and recovery plans but can be difficult to obtain for threatened and endangered species. The simple, field-ready methods described in this article can easily be adapted or modified to suit ongoing monitoring programs of sturgeon and can be used to improve population assessments for these data-limited species.

Conclusions

The second marginal pectoral-fin ray provided an excellent alternative to fin spines for aging Gulf sturgeon. Removal was minimally deleterious, and the banding pattern was generally clear and consistent. Annual band formation was validated for 3 Gulf sturgeon with 6, 7, and 8 band pairs, but 10 mg/kg is too low a dose of OTC to reliably mark calcified structures of Gulf sturgeon. A dose of 25 mg/kg is recommended for future validation studies. The methods described in this article have wide applicability to other sturgeon species and are important for population assessment of these overexploited fishes.

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Release mortality in the red snapper (*Lutjanus campechanus*) fishery: a meta-analysis of 3 decades of research

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Abstract—The value of catch-and-release fishing as a conservation measure is highly dependent upon rates of discard frequency and release mortality. Therefore, it is important to understand how estimates of these variables are affected by factors such as capture depth and water temperature. The meta-analytical approach to modeling used here for red snapper (*Lutjanus campechanus*) in the Gulf of Mexico provides a robust method for dealing with study-specific differences in experimental protocols and for estimating release (discard) mortality as a function of key factors. Results of this analysis showed significant increases in mortality by depth and for the commercial sector. The most consistent result was the positive correlation between depth and estimates of release mortality, a relationship that was present regardless of study method, fishing sector, hook type used, or season of study. The effect of venting (deflating the swim bladder by puncture) was dependent on whether the study produced estimates of immediate or delayed mortality. Immediate estimates indicated that mortality rates are lowered by venting whereas delayed estimates indicated that venting increased mortality rates. This result is largely reflective of the use of submergence ability, from surface-release studies, as a proxy for mortality. The model's interaction result indicates that recompression of fish may be a viable alternative to venting and that, if a recompression device is not available, venting at least improves the likelihood that a fish can submerge and return to protective habitat. The depth-based functional relationships developed in this model were used in the most recent red snapper stock assessment in 2012, and that use was a change from previous assessments where region-specific point estimates were used.

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The use of catch-and-release (CAR) fishing as a conservation measure began in Great Britain during the 19th century, eventually was practiced in the United States voluntarily by the early 1900s, and was used first as a management tool in salmonid fisheries in the 1950s (Policansky, 2002). Since that time, it has been practiced successfully to promote sustainable fisheries and is widely accepted by many anglers as a wise conservation strategy. The intent behind CAR regulations is to reduce fishing mortality for important age groups of fish, often to allow young ages to grow and mature to reproductive ages or to protect spawning adults. Catch-and-release requirements in the red snapper (*Lutjanus campechanus*) fishery have expanded in recent years to encompass the release of all size classes during seasonal (or longer) closures in response to fishery management plans required by the Magnuson-Stevens Fishery Conservation and Management Act and which place strict limits on total removals, including those in open-access recreational fisheries.

Despite the intent of CAR fishing regulations, for many species, stress from capture can lead to barotrauma injuries and reduced reflex responses that result in increased release mortality and render some CAR measures ineffective (Campbell et al., 2010a; Davis, 2010). Stressors experienced by fish during CAR fishing can include hooking trauma, physical overexertion, barotrauma, rapid thermal change, air exposure, and physical handling (Davis et al., 2001; Rummer and Bennett, 2005; Nieland et al., 2007; Jarvis and Lowe, 2008). These stressors from CAR fishing may also translate into long-term, sublethal, negative consequences, such as reduced growth and fecundity (Woodley and Peterson, 2003; Ryer et al., 2004; Davis, 2007). The effects of CAR fishing can be problematic particularly for marine species like red snapper that inhabit relatively deep water and possess a physoclis-

tous gas bladder (i.e., an air bladder not connected to the alimentary canal).

Red snapper have been fished in the Gulf of Mexico (GOM) for more than a century and compose the most economically important fishery in this region (Strelcheck and Hood, 2007). The first regulations managing the fishery were put in place in 1984 in response to the overfished status of the stock (for a comprehensive management history, see Hood et al., 2007). In general, management has focused on annual time closures and minimum-size regulations that have increased the number of regulatory discards, particularly in open-access recreational fisheries. Management of commercial fisheries has shifted recently to an annual catch share system, which removed the necessity to discard fish during seasonal closures but still does not eliminate regulatory discards if vessels do not possess catch shares or target other reef-associated species after annual catch shares are exhausted.

The focus of management efforts has also shifted to regulations intended to reduce or minimize discard mortality. Regulations adopted in 2008 in the GOM, for example, require commercial and recreational fishermen to use circle hooks and to use a venting tool when catching reef fish. Venting tools are used to puncture and deflate the swim bladder after fish are rapidly retrieved as a means to mitigate the effects of barotrauma. Recent research indicates that circle hooks are beneficial for reducing potentially fatal injuries for reef fish caught with hook-and-line gear, particularly for red snapper (Sauls and Ayala, 2012). A meta-analysis of the relationship between venting practices and release mortality indicated negligible effects of venting for red snapper (Wilde, 2009). In the Wilde (2009) meta-analysis, 1 experiment showed positive effects of venting on survival (Gitschlag and Renaud, 1994), 2 reports showed neutral results (Render and Wilson, 1994; Render and Wilson, 1996), and 1 experiment showed a negative effect (Burns et al.[1]).

Regulatory discards account for an increasing portion of total catch for managed reef fish in fisheries across the United States, and, in the U.S. GOM, the ratio of discards to landings for directed reef fish fisheries was estimated at 41% (Harrington et al., 2005). The rate at which fish are discarded and quantification of their fate after release are crucial data needs for regional stock assessments in the GOM and south Atlantic. Because of the wide range in reported mortality rates (SEDAR[2]; Campbell et al.[3]) and the confounding nature of the potential interacting factors, a comprehensive evaluation of pertinent research is needed.

Each method used to derive mortality estimates has its benefits, biases, and shortcomings that require exploration; however, in general, problems are associated with the timing of observation, exclusion of predation, insufficient tag returns, sample size, and artifacts of experimental protocols (Campbell et al., 2010b). Methods used to derive estimates in the red snapper fishery include surface-release observation, caging studies, hyperbaric-chamber simulations, acoustic tagging, and passive tagging (Table 1, Fig. 1). Mortality estimates from studies (Table 1) with the use of these methods are broadly categorized as either immediate (seconds to minutes) or delayed (hours to days) and refer to the time span over which the estimate is calculated. These different types of experiments and, therefore, different types of estimates are often treated as equivalents when used in an assessment. Although this aggregate approach is pragmatic, it may result in the use of imprecise estimates and introduce unexplored or unknown sources of bias.

We present a meta-analytical approach with the intent of identifying critical issues and deriving a model of release mortality in the red snapper fishery of the GOM as a function of important covariates, such as depth, season, and capture gear. Meta-analytical methods allow inclusion of all available point estimates, include a sample-size weighting scheme, and allow for the use of covariates in a mixed-effects modeling approach (Viechtbauer, 2010). The meta-analytical approach was developed, and is useful, because it reduces the introduction of bias that hinders nonparametric approaches often found in review papers (Sterne et al., 2000; Nakagawa and Santos, 2012).

Materials and methods

Data used in this meta-analysis were compiled from 11 studies that produced 75 distinct estimates of release mortality (Table 1). These studies represent everything the release mortality working group was aware of in 2012 in preparation for the Southeast Data, Assessment, and Review (SEDAR) of Gulf of Mexico red snapper, the SEDAR 31 stock assessment. The data workshop portion of the SEDAR process typically consists of analysis by expert panelists and assessment biologists of data sources that potentially feed into stock assessment models (for further information about SEDAR, visit: http://www.sefsc.noaa.gov/sedar/).

There are multiple estimates from some of the 11 studies examined for this meta-analysis because they were conducted at multiple water depths or seasons. Most of the estimates were compiled from refereed pub-

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Table 1

List of studies (Study) used in a meta-analysis of release mortality of red snapper (*Lutjanus campechanus*) in the Gulf of Mexico for which estimates (Mort) are categorized by 5-m-depth groups, study type (Type), timing of the estimate (Timing), fishing sector (Sector), season conducted (Season), hook type used (Hook), frequency of venting (Vent), and sample size (*n*). Study types: surface release (SR), caging (C), passive tagging (PT), and acoustic tagging (AT). Timing of estimates: immediate (I) and delayed (D). Sector: recreational (Rec) and commercial (Com). Season: summer (Sum), spring (Spr), winter (Win), Fall (Fall), and annual (Ann). Hook: circle hooks (C), j-hooks (J), and mixed hooks (M, both j and circles used). Venting: venting (V) and nonventing (NV).

Depth	Mort	Type	Timing	Sector	Season	Hook	Vent	*n*	Study
10	0.280	SR	I	Rec	Sum	J	NV	25	Dorf, 2003
10	0.700	SR	I	Com	Win	C	NV	40	Nieland et al., 2007
15	0.282	SR	I	Rec	Sum	J	NV	425	Dorf, 2003
20	0.273	SR	I	Rec	Sum	J	NV	825	Dorf, 2003
20	0.252	SR	I	Com	Win	C	NV	465	Nieland et al., 2007
21	0.090	SR	I	Rec	Ann	J	V	1064	Patterson et al., 2001
22	0.210	C	D	Rec	Ann	J	NV	14	Parker[5]
24	0.010	SR	I	Rec	Fall	J	V	140	Gitschlag and Renaud, 1994
25	0.200	C	D	Rec	Ann	J	V	282	Render and Wilson, 1996
25	0.410	SR	I	Rec	Sum	J	NV	525	Dorf, 2003
25	0.280	PT	D	Rec	Sum	C	V	353	Sauls[8]
25	0.260	PT	D	Rec	Sum	C	V	353	Sauls[8]
25	0.230	PT	D	Rec	Fall	C	V	353	Sauls[8]
25	0.160	PT	D	Rec	Fall	C	V	353	Sauls[8]
25	0.290	PT	D	Rec	Win	C	V	353	Sauls[8]
25	0.250	PT	D	Rec	Win	C	V	353	Sauls[8]
25	0.170	PT	D	Rec	Spr	C	V	353	Sauls[8]
25	0.180	PT	D	Rec	Spr	C	V	353	Sauls[8]
27	0.140	SR	I	Rec	Ann	J	V	856	Patterson et al., 2001
30	0.110	C	D	Rec	Ann	J	NV	30	Parker[5]
30	0.100	SR	I	Rec	Fall	J	V	31	Gitschlag and Renaud, 1994
30	0.420	C	D	Rec	Sum	M	V	47	Diamond and Campbell, 2009
30	0.130	C	D	Rec	Fall	M	V	30	Diamond and Campbell, 2009
30	0.470	SR	I	Rec	Sum	J	NV	225	Dorf, 2003
30	0.213	SR	I	Rec	Fall	C	V	137	Campbell et al., 2010a
30	0.227	SR	I	Rec	Sum	C	V	137	Campbell et al., 2010a
30	0.030	SR	I	Rec	Win	J	V	138	Patterson[4]
30	0.060	SR	I	Rec	Spr	J	V	31	Patterson[4]
30	0.070	SR	I	Rec	Sum	J	V	52	Patterson[4]
30	0.120	SR	I	Rec	Fall	J	V	221	Patterson[4]
30	0.681	SR	I	Com	Win	C	NV	789	Nieland et al., 2007
32	0.180	SR	I	Rec	Ann	J	V	1012	Patterson et al., 2001
35	0.150	SR	I	Rec	Sum	J	NV	100	Dorf, 2003
35	0.040	SR	I	Rec	Win	J	V	375	Patterson[4]
35	0.100	SR	I	Rec	Spr	J	V	196	Patterson[4]
35	0.130	SR	I	Rec	Sum	J	V	264	Patterson[4]
35	0.170	SR	I	Rec	Fall	J	V	563	Patterson[4]
35	0.370	PT	D	Rec	Sum	C	V	863	Sauls[8]
35	0.330	PT	D	Rec	Sum	C	V	863	Sauls[8]
35	0.280	PT	D	Rec	Fall	C	V	863	Sauls[8]
35	0.220	PT	D	Rec	Fall	C	V	863	Sauls[8]
35	0.220	PT	D	Rec	Win	C	V	863	Sauls[8]
35	0.120	PT	D	Rec	Win	C	V	863	Sauls[8]
35	0.230	PT	D	Rec	Spr	C	V	863	Sauls[8]
35	0.210	PT	D	Rec	Spr	C	V	863	Sauls[8]
40	0.440	SR	I	Rec	Fall	J	V	61	Gitschlag and Renaud, 1994
40	0.400	SR	I	Rec	Sum	J	NV	155	Dorf, 2003
40	0.050	SR	I	Rec	Win	J	V	65	Patterson[4]
40	0.160	SR	I	Rec	Spr	J	V	107	Patterson[4]

Table continued

Table 1 continued

Depth	Mort	Type	Timing	Sector	Season	Hook	Vent	*n*	Study
40	0.160	SR	I	Rec	Sum	J	V	44	Patterson[4]
40	0.200	SR	I	Rec	Fall	J	V	60	Patterson[4]
40	0.420	C	D	Rec	Sum	M	V	56	Diamond and Campbell, 2009
40	0.340	C	D	Rec	Fall	M	V	32	Diamond and Campbell, 2009
40	0.740	SR	I	Com	Win	C	NV	814	Nieland et al., 2007
45	0.630	SR	I	Rec	Sum	J	NV	280	Dorf, 2003
50	0.360	C	D	Rec	Fall	J	V	55	Gitschlag and Renaud, 1994
50	0.690	C	D	Rec	Sum	M	V	24	Diamond and Campbell, 2009
50	0.440	C	D	Rec	Fall	M	V	36	Diamond and Campbell, 2009
50	0.610	SR	I	Rec	Sum	J	NV	105	Dorf, 2003
50	0.790	AT	D	Rec	Sum	M	V	24	Diamond et al.7
50	0.400	AT	D	Rec	Win	M	V	20	Diamond et al.7
50	0.744	SR	I	Com	Win	C	NV	1638	Nieland et al., 2007
55	0.580	SR	I	Rec	Sum	J	NV	240	Dorf, 2003
60	0.380	SR	I	Rec	Sum	J	NV	125	Dorf, 2003
60	0.214	SR	I	Rec	Fall	C	V	282	Campbell et al., 2010a
60	0.258	SR	I	Rec	Sum	C	V	282	Campbell et al., 2010a
60	0.694	SR	I	Com	Win	C	NV	464	Nieland et al., 2007
65	0.370	SR	I	Rec	Sum	J	NV	50	Dorf, 2003
70	0.330	SR	I	Rec	Sum	J	NV	10	Dorf, 2003
70	0.782	SR	I	Com	Win	C	NV	404	Nieland et al., 2007
75	0.230	SR	I	Rec	Sum	J	NV	75	Dorf, 2003
80	0.470	SR	I	Rec	Sum	J	NV	100	Dorf, 2003
80	0.886	SR	I	Com	Win	C	NV	88	Nieland et al., 2007
90	0.912	SR	I	Com	Win	C	NV	68	Nieland et al., 2007
95	0.560	SR	I	Rec	Sum	J	NV	30	Dorf, 2003

lications (Gitschlag and Renaud, 1994; Render and Wilson, 1994; Patterson et al., 2001; Dorf, 2003; Nieland et al., 2007; Diamond and Campbell, 2009; Campbell et al., 2010a). One assessment was calculated from unpublished data (Patterson[4]), and 5 estimates were available only from gray literature (Parker[5]; Burns et al.[6]; Diamond et al.[7]; Sauls[8]). Data extracted from each publication included proportional mortality, water depth (in meters), study type (surface release, caging, passive tagging, acoustic tagging, or hyperbaric chamber), timing of the mortality estimate (immediate or delayed), fishing sector evaluated (commercial or recreational), season (winter, spring, summer, fall, or annual), hook type used (circle, J, or mixed), venting treatment (venting or nonventing), and sample size (*n*).

Several discrepancies about release mortality rates reported in the literature were found. The 10-, 15-, 20-, and 25-m depth groups from Dorf (2003) appeared to be aggregated and reported as a single estimate for one depth group (21–25 m) in a previous stock assessment in 2005 (SEDAR[2]). The 30-, 40-, and 50-m values from Diamond and Campbell (2009) also were aggregated and reported as annual estimates in the previous assessment in 2005 (SEDAR[2]). Because there is uncertainty about why these 2 data sets were aggregated in the previous assessment, our meta-analysis relied on published values as being representative of those works. The only data set from a commercial fishery that we found was that of the Nieland et al. (2007) study. This lone commercial-fishery study comprised data over 4 years at more than 273 separate fishing sites, the majority of which were located in coastal Louisiana. Nieland et al. (2007) originally reported site-specific estimates of release mortality, many of which had small sample sizes (*n*=~5–10). Therefore, mortality

[4] Patterson, W. 2011. Unpubl. data. Univ. South Alabama, Mobile AL 36688.

[5] Parker, R. O. 1985. Survival of released red snapper progress report. SEDAR24-RD12, 9 p. [Available from http://www.sefsc.noaa.gov/sedar/.]

[6] Burns, K. M., R. R. Wilson Jr., and N. F. Parnell. 2004. Partitioning release mortality in the undersized red snapper bycatch: comparison of depth vs. hooking effects. Mote Marine Laboratory Technical Report No. 932, 43 p. [Available from Mote Marine Laboratory, 1600 Ken Thompson Pkwy., Sarasota, FL 34236.]

[7] Diamond, S. L., T. Hedrick-Hopper, G. Stunz, M. Johnson, and J. Curtis. 2011. Reducing discard mortality of red snapper in the recreational fisheries using descender hooks and rapid recompression. Final report, grant no. NA07NMF4540078, 52 p. [Available from http://www.sefsc.noaa.gov/P_QryLDS/download/CR262_Diamond_2011.pdf?id=LDS.]

[8] Sauls, B. 2012. Release mortality estimates for recreational hook-and-line caught red snapper derived from a large-scale tag-recapture study in the eastern Gulf of Mexico. SEDAR31-DW23, 21 p. [Available from http://www.sefsc.noaa.gov/sedar/.]

rates were recalculated for discrete depth groups from the original data by aggregating sites by depth.

Ideally, the frequency at which fish were vented would be calculated; however, some studies reported that venting occurred irregularly and at the choice of participants. If a study reported at least some amount of venting, then it was categorized as a venting treatment; otherwise, it was considered a nonventing treatment. Caging studies that reported a venting treatment were maintained as reported, but it should be noted that those experiments included recompression of fish (i.e., their air bladders) by submergence back to depth in cages regardless of whether a fish had been vented. Because few studies reported hook size, it was not included. Finally, the intent of this meta-analysis was to evaluate release mortality under normal fishing conditions; therefore, estimates from the hyperbaric-chamber study (Burns et al.[6]) were not included in our study.

The meta-analytical model used in our study is a special case of a weighted general linear model as detailed in the metafor package (Viechtbauer, 2010), a meta-analysis package for R software. The analysis was performed on effect size (*es*) rather than on raw proportions, where *es* was the logit-transformed proportion and was calculated with the following equation:

$$es = \log\left(\frac{x_i}{(n_i - x_i)}\right), \quad (1)$$

where x_i = the total number of individuals that experienced mortality; and
n_i = the total sample size.

The estimate and the corresponding sampling variance were calculated by using the escalc function in the metafor package (Viechtbauer, 2010) in R software, vers. 2.15.1 (R Core Team, 2012).

We fitted estimates of effect size in a mixed-effects model to evaluate the effects of depth, fishing sector, timing of the mortality estimate, venting treatment, season, and hook type (Viechtbauer, 2010). For the categorical variables, the absence of group membership (i.e., setting that value to 0) by default defines the opposite group; therefore, there is no need to have all variables included. For instance, identifying estimates associated with commercial data as 1 automatically defines values set equal to 0 as being associated with recreational estimates. The full estimated model is shown below:

$$Prb\ (mortality) \sim depth + sector + timing + venting + season + hook\ type + rate + timing{*}venting,$$

where *depth* of capture in meters is modeled as a continuous variable and all other variables are modeled as categorical. *Sectors* were defined as commercial or recreational. *Timing* was defined as either immediate mortality or delayed mortality, referred to hereafter simply as immediate or delayed. *Venting* treatments included venting and nonventing. *Season* variables included spring, summer, fall, or winter. *Hook types* were tested as circle or as J- and mixed hooks combined because we were interested in the effect of circle hook regulations. The *rate* variable represents each individual estimate and was modeled as a random effect (i.e. estimated mortality rate). Therefore, the model treated multiple estimates coming from a single study as unique estimates from the available population. Treatment of multiple estimates from the same study as unique estimates occurred when a study was conducted over different seasons or over a range of depths. Finally, because we wanted to test whether the *venting* treatment was confounded with the study type and timing of the estimate (immediate), we also included an interaction term (*timing*venting*).

Several additional model runs were performed to evaluate sensitivity of the model to various issues. The commercial data set was represented by a single study and, although it was a fairly extensive study that produced many estimates, it may not be representative of all commercial fisheries for red snapper. Therefore, we made model runs that excluded the data from Nieland et al. (2007).

Heterogeneity (τ^2) was estimated by using restricted maximum-likelihood. Coefficients for μ, $\beta_0,\ldots,\beta_p$ then were estimated with weighted least squares in which each estimate of effect size was weighted by the inverse of its variance. Wald-type tests and confidence intervals were calculated for μ, $\beta_0,\ldots,\beta_p$, assuming normality. On the basis of the fitted model, we calculated predicted values and residuals. Cochran's *Q*-test was used to assess the amount of heterogeneity among studies (i.e., a null hypothesis of $\tau^2=0$). Model predictions were calculated with the predict function in the metafor package. The predict function allows for the input of a range of values (e.g., depths) over which to calculate model predictions and also allows for the adjustment of coefficient weights so that individual treatment effects can be isolated (e.g., venting and season). Predicted values and associated upper and lower bounds were then converted back to proportions by taking the inverse of the logit-transformed effect-size data with the following equation:

$$Proportion = \frac{exp^{es}}{\left(1 + exp^{es}\right)}, \quad (2)$$

Average model predictions were evaluated by giving equal weighting to the coefficients within fishing sector, timing of mortality, venting, season, and hook type and by inputting a depth range of 10–100 m. Model predictions for the various venting and season treatments were then calculated through adjustment of the weighting scheme submitted to the predict function. For instance, to evaluate the effect of 100% venting, all of the weight for the venting treatments was put onto the treatment with 100% venting, and model predictions were recalculated.

Results

Estimates of release mortality ranged from 0% to 91% over the collection of studies evaluated (Table 1, Fig. 1). The lowest estimate of 0% was associated with a hyperbaric-chamber study (Burns et al.[6]), and the highest value of 91% was associated with the only data available for the commercial fishing sector. The most common method used in these studies was surface release, followed by passive tagging, caging, hyperbaric chamber, and acoustic tagging (Table 1, Fig. 1). Release estimates were calculated most frequently from summer data, followed by winter, fall, spring, and annual (Table 1). Studies were conducted primarily at depths that ranged between 20 and 50 m; infrequent representation was found outside that range (Fig. 2). Estimates mainly were associated with the recreational fishing sector or with methods and gear commonly used in recreational fishing. Commercial fishing data were represented by a single study. Estimates of hook type were fairly balanced between the studies that used J-hooks and the ones that used circle hooks; fewer studies used a mixture of hook types. Studies that used a mixture of hook types were associated most commonly with those studies that used direct observations in the fishery and for which gear choice was, therefore, reflective of common fishing practices. Studies in which a venting treatment was used 100% of the time always were associated with controlled scientific experiments (i.e., they did not involve direct observations of the fishery). Regardless of the fishing sector, nonventing estimates were associated most frequently with studies where fishing practices were observed from working vessels.

Results of the meta-analysis of the full complement of data showed significant effects for the following coefficients: intercept, depth, sector, timing*venting interaction, winter, and spring (Table 2). The timing, venting, fall, annual, and hook-type effects were nonsignificant. The amount of heterogeneity in effect size from the mixed-effects model was estimated to be 0.31. Cochran's Q-test for the mixed-effects model also showed significant residual heterogeneity (Q_E=663.20, df=64, P<0.0001), indicating that the model did not fully explain the observed variation in estimates of release mortality. Depth was the most important factor determining release mortality and consistently showed a positive relationship with mortality (Figs. 3–6). Model

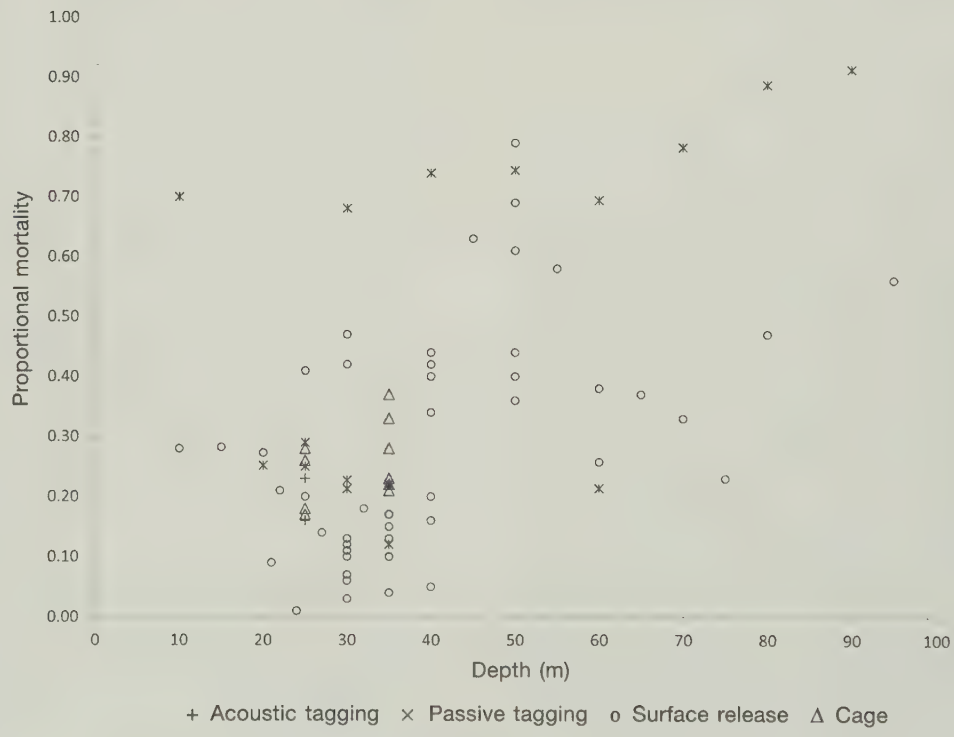

Figure 1

Scatter plot of estimates (proportions) of release mortality by depth (0–100 m) for red snapper (*Lutjanus campechanus*) in the Gulf of Mexico determined from 11 studies of 4 types: acoustic tagging (+), passive tagging (*), surface release (o), and caging (Δ). The values represented in this plot were used in the meta-analytical model of this study and are also included in Table 1.

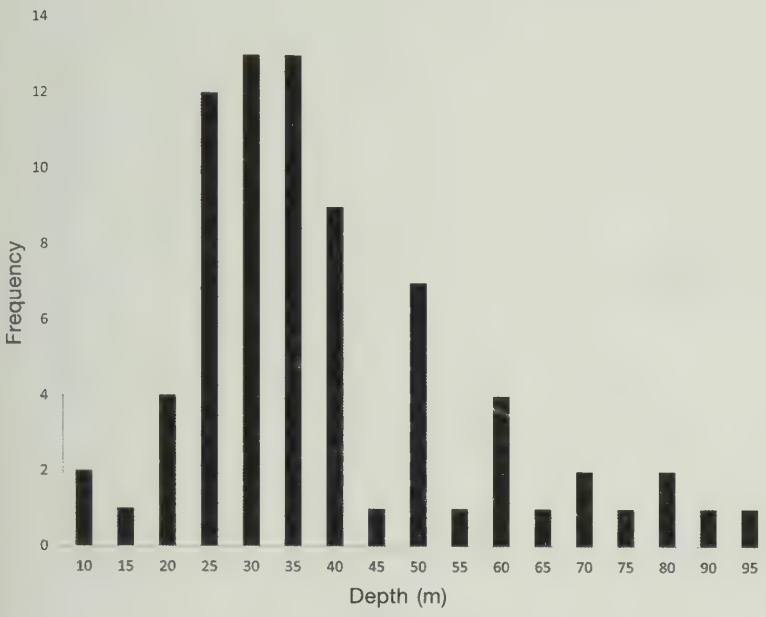

Figure 2

Depth-frequency distribution associated with estimates (n=75) of release mortality for red snapper (*Lutjanus campechanus*) in the Gulf of Mexico. Values were compiled from 11 studies and were included in the meta-analytical model developed in this study.

coefficients indicated that the commercial fishing sector was the most influential factor that increased mortality and that the interaction and winter terms were the most influential factors that reduced mortality. That the term for the timing*venting interaction was statistically significant indicates that the effect of venting was dependent on the timing of the estimate and, therefore, indicates that immediate measurements of mortality (e.g., surface-release methods) were affected significantly decreased by venting. The venting coefficient was positive, indicating that, for delayed estimates, venting would increase mortality.

Removal of estimates of release mortality in the commercial sector had little effect on model outcomes (i.e., significant coefficients) compared with model runs that included that data. The amount of heterogeneity in effect size from the mixed-effects model without commercial-sector data was estimated to be 0.29. Cochran's Q-test for the mixed-effects model also showed significant residual heterogeneity (Q_E=440.81, df=56, P<0.0001), indicating that the model did not fully explain the observed variation in release mortality estimates. Model coefficients indicated that depth was the most influential factor that increased mortality and that the winter and spring seasonal conditions were important in reduction of mortality. Significant effects in this second model included the following coefficients: intercept, depth, timing*venting interaction, winter, and spring (Table 3). Timing, venting, fall, annual, and hook-type effects were nonsignificant. Conservation of the results despite removal of the commercial data indicated that the model was largely driven by recreational data.

Predicted rates of discard mortality by depth, mode, and season were derived by weighting model coefficients generated through the use of the full complement of data (i.e., commercial data were included). Average predictions and treatment-specific predictions were generated by weighting the coefficients accordingly. Over a depth range of 10–100 m, average model predictions (equal weighting of coefficients) of release mortalities ranged between 19% and 67% (Fig. 3; Table 4). As indicated by the statistical significance of the timing*venting interaction, the predicted rates of release mortality by venting treatments were dependent on whether a study relied on the surface-release method; this method produces immediate estimates, and other methods result in delayed estimates (Fig. 4). Immediate estimates indicated that venting decreased mortality rates, but delayed estimates indicated that venting increased mortality rates. Regardless of the model run, the winter and spring terms were significant and the predicted release mortality rates were

Table 2

Model coefficients, standard errors of the mean (SE), and P-values estimated in a meta-analysis of release mortality of red snapper (*Lutjanus campechanus*) in the Gulf of Mexico. This meta-analysis included both recreational and commercial data. Significant coefficients are highlighted with asterisks (*** P<0.0001, ** P<0.001, * P<0.05), and "ns" indicates nonsignificant coefficients.

Data type	Coefficient	SE	P	Significance
Intercept	−1.9136	0.7085	0.0069	**
Depth	0.0209	0.0046	<.0001	***
Sector	2.2769	0.4228	<.0001	***
Timing	0.5304	0.7009	0.4492	ns
Venting	0.6955	0.6732	0.3016	ns
Timing*Venting	−1.4858	0.7419	0.0452	*
Winter	−0.9905	0.2789	0.0004	***
Spring	−0.7701	0.2872	0.0073	**
Fall	−0.3364	0.235	0.1524	ns
Annual	−0.3668	0.3522	0.2977	ns
Hook type	0.0139	0.2107	0.9472	ns

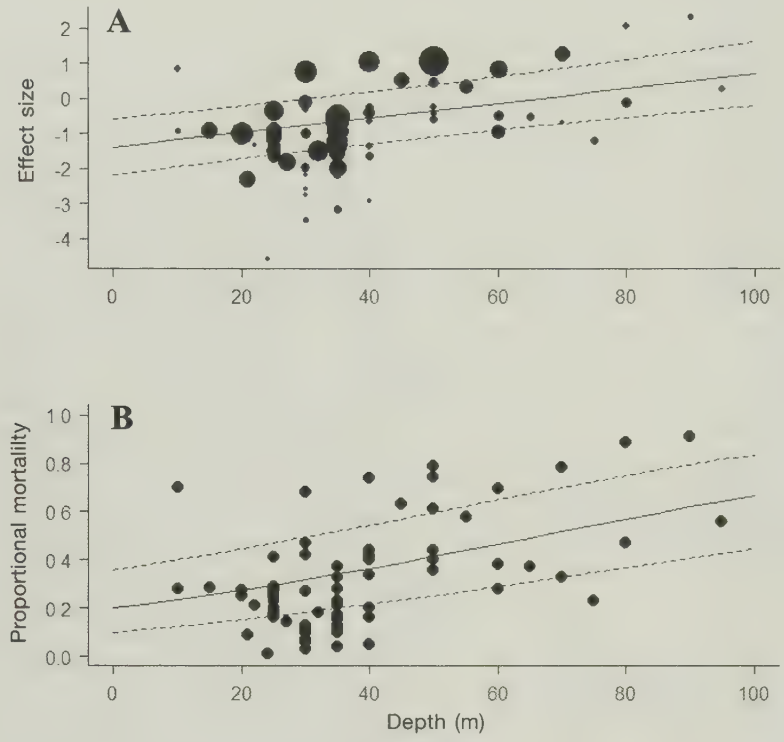

Figure 3

Predicted relationship between depth and release mortality of red snapper (*Lutjanus campechanus*) in the Gulf of Mexico derived from the meta-analytical model in which coefficients were equally weighted to produce the predicted values (average model). (**A**) Relationship plotted with effect-size data weighted by the inverse of its variance; dot size, then, is proportional to the inverse of the corresponding sampling variance, and increasing dot size indicates better fit to the model. (**B**) Same relationship plotted with effect size data converted back to original proportions to better visualize predictions of release mortality.

reduced during those seasons (Fig. 5; Table 4). Inclusion of the commercial data in the model indicated that release mortality rates were significantly higher in the commercial fishing sector than in the recreational fishing sector (Fig. 6; Table 4).

Discussion

The mixed-effects modeling approach in which a random effect is estimated for each individual study, while estimating discard mortality as a function of key factors of interest, provides a robust method for dealing with specific differences due to either experimental protocols or other factors. The functional relationships developed in this model had been used in the most recent red snapper stock assessment in 2012, and that use was a change from previous assessments based on region-specific point estimates and that did not provide an estimate a depth-related function (Campbell et al.[3]). The various differences in experimental protocols represented nuisance factors for the estimation of a range of discard mortality rates by depth. Nonetheless, significant residual heterogeneity was observed in the model, indicating that there likely were other unquantified variables that influence release mortality. Results from the various studies have to be evaluated within the context of the experimental methods used, but overall our meta-analytical model resulted in consistent results that isolated important factors for release mortality in the red snapper fishery.

There was a consistent, positive correlation between depth and release mortality estimates regardless of venting treatment, season, or fishing sector (Tables 1–4; Figs. 3–6). Presence of a positive correlation between depth and mortality is frequently reported in the literature, and the relationship is thought to be associated primarily with injuries sustained during decompression, such as overexpansion and rupture of the gas bladder, esophageal eversion, cloacal prolapse, exophthalmia, and gas infusion into vital organs (Davis, 2002; Rummer and Bennett, 2005; Hannah et al., 2008). The development of a predictive relationship with depth is important because previous stock assessments of red snapper, completed before this model, were based on single estimates that were fixed by region (i.e., east and west GOM) rather than on a depth relationship. Still, although depth was a consistent factor for the explanation of release mortality, the results from the studies examined were complicated by study-specific experimental methods.

Estimated rates of release mortality were significantly higher for the commercial sector than for the recreational sector, but, unfortunately, they were derived from a single surface-release study that was conducted in a single region (Nieland et al., 2007). Further complicating the commercial data was the fact that no fish were vented before release in that study. Comparable surface-release studies of the recreational sector revealed that at least some amount of venting occurred, and all of those studies resulted in lower estimates of release mortality (Patterson et al., 2001; Dorf, 2003; Campbell et al., 2010a, Patterson[4]). Because commercial fishing operations were observed directly in the Nieland study, the estimates are at least reflective of common venting practices for that fishing sector at that time and region. Impor-

tantly, our meta-analysis indicates that those estimates are highly dependent on the frequency that fish were vented before release (Fig. 4). The accuracy of surface-release estimates is tenuous because misclassification of fate after release of tagged fish can be high; therefore, significant investigation into the rate of misclassification is needed (Sauls, 2014).

Alternatively, it is possible that commercial fishing gear and practices may contribute to elevated estimates of release mortality for that sector. In the commercial sector, either electric or hydraulic bandit-gears with multiple hooks rapidly retrieve fish from depth. Rapid ascent and increased catch rates potentially result in increased sorting, handling, and air-exposure times, all of which have been shown to increase release mortality (Davis, 2002). The commercial data set in this meta-analysis may not be completely representative of that fishing sector, but the assessment process by rule is required to use the best available data to make decisions.

Finally, results of our analysis did not change after the removal of the commercial data set (Tables 2 and 3), indicating that model outcomes and predictions were driven by the recreational estimates and likely are most representative of that sector. Research clearly is needed to sort out true effects associated with commercial fishing from more general effects that are seen throughout the fishery and that are confounded by estimation methods (i.e., surface-release studies).

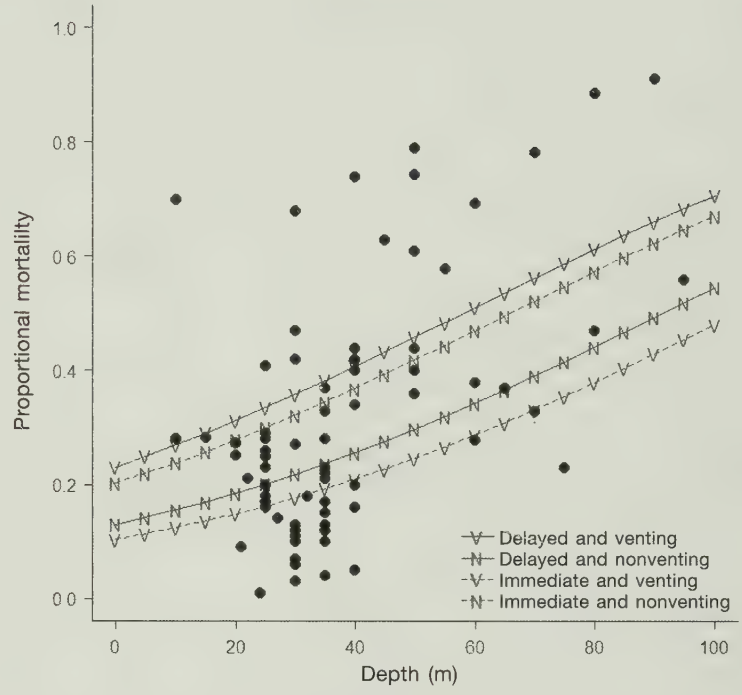

Figure 4

Predicted relationship between depth and release mortality (proportion) of red snapper (*Lutjanus campechanus*) in the Gulf of Mexico derived from the meta-analytical model for the interactions of immediate (dashed lines; seconds to minutes) and delayed (solid lines; hours to days) timing of mortality estimates with the venting (V) and nonventing (NV) treatments. Immediate estimates of release mortality indicate that venting decreases mortality, but delayed estimates indicate that venting increases mortality.

Table 3

Model coefficients, standard errors of the mean, and *P*-values estimated in a meta-analysis of release mortality of red snapper (*Lutjanus campechanus*) in the Gulf of Mexico. This meta-analysis excluded the commercial data set. Significant coefficients are highlighted with asterisks (*** $P<0.0001$, ** $P<0.001$, * $P<0.05$), and "ns" indicates nonsignficant coefficients.

	Coefficient	SE	*P*	Significance
Intercept	−1.782	0.706	0.0116	*
Depth	0.0176	0.0054	0.0012	**
Timing	0.5542	0.688	0.4205	ns
Venting	0.6858	0.6611	0.2996	ns
Timing*Venting	−1.4951	0.7273	0.0398	*
Winter	−0.9974	0.2715	0.0002	***
Spring	−0.7833	0.2794	0.0051	**
Fall	−0.3397	0.2287	0.1375	ns
Annual	−0.4132	0.3446	0.2305	ns
Hook type	0.0023	0.2054	0.9912	ns

The significant interaction between the immediate timing of estimates and venting treatments indicates that the effect of venting on rates of release mortality was dependent on the timing component inherently incorporated into study-specific methods (i.e., surface-release studies produce immediate estimates). Estimates were available most commonly from surface-release studies (Table 1; Fig. 1) and those are contingent on the assumption that failure of a fish to submerge is reflective of mortality (Campbell et al., 2010a). Immediate estimates were significantly lower for the venting treatment than for the nonventing treatment (Fig. 4). For the delayed estimates (e.g., mortality estimates from caging studies), venting increased rates of release mortality— a finding that is congruent with results presented in a separate meta-analysis of venting from the same set of studies that produced delayed estimates (Wilde, 2009). Surface-release studies likely are too limited in scope to correctly evaluate the long-term effects of venting on release mortality. Furthermore, sur-

face-release studies also inject uncertainty into assessment models because of the unknown misclassification rates that result from the use of submergence as a proxy for mortality. Submergence data should be collected and used only as a last resort in the estimation of release mortality.

Both model runs, with and without the data from the commercial fishing sector, indicated that venting was not significant—a finding that agrees with the conclusions in Wilde (2009) that there was no evidence for an effect of venting. Two of the studies that produced delayed estimates specifically tested the effects of venting after fish were submerged in cages to at least 2 atm of pressure (Gitschlag and Renaud, 1994; Render and Wilson, 1994). Submergence to 2 atm halves gas volume in the air bladder and effectively recompresses the fish. Both of these caging studies reported no difference in survival by venting treatments, but neither study addressed the issue of recompression. Recompressing the gas bladder may have had the same effect as venting the fish and, perhaps, explains the lack of a difference in survival between venting treatments. Ultimately, these caging studies lend insight in regard to venting versus recompression, but they are not reflective of day-to-day fishery operations in which fish are released at the surface.

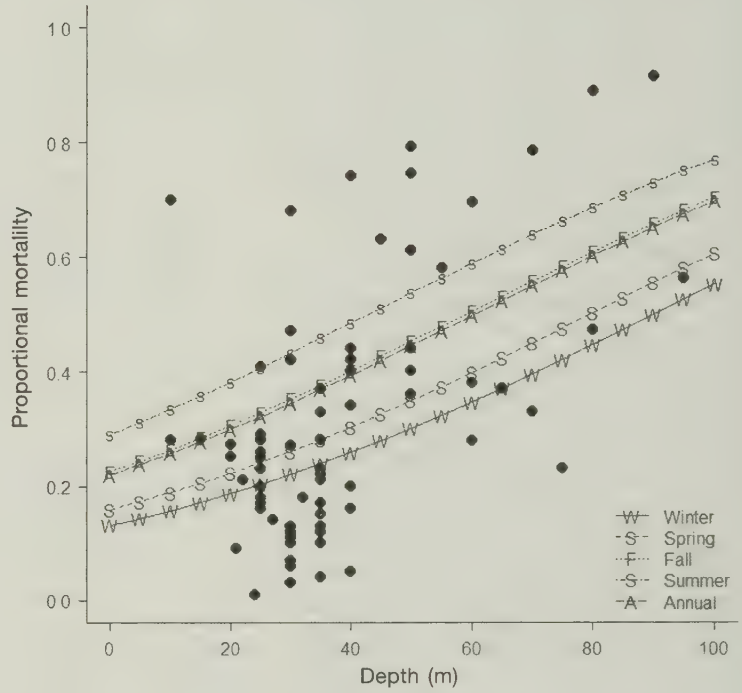

Figure 5

Predicted relationship between depth and release mortality (proportion) of red snapper (*Lutjanus campechanus*) in the Gulf of Mexico derived from the meta-analytical model for the winter, spring, summer, and fall seasons and annual classification. Results indicate that cooler seasons, and therefore water temperature, reduces release mortality.

Venting is best evaluated with tag-and-recapture studies in which fish are released as they would be in regular fishery operations. The only tag-and-recapture study that directly compared venting treatments, and that was available for inclusion in either meta-analysis, simply evaluated recapture rates and did not generate a model for estimation of release mortality (Burns et al.[1]). Furthermore, estimates from the Burns et al.[1] tag-and-recapture study did not account for spatial issues; nor did it incorporate fishing effort, making interpretation of the results problematic. Recently developed tag-and-recapture models from other fisheries that use fishery-dependent data and incorporate fishing effort, hook type, and venting procedures into the estimates should provide a more robust method to test venting (Hueter et al., 2006; Sauls and Ayala, 2012; Sauls, 2014). The interaction result indicates that recompression of fish may be a viable alternative to venting, but if a recompression device is not available, then venting at least improves the likelihood that a fish can submerge and return to protective habitat.

Recent research in the red snapper fishery has been focused on bottom-release devices, 2 of which have been tested experimentally. The use of a bottom-release device is similar to venting in that the goal is to reverse the effects of barotrauma, but instead of deflating the bladder by puncture it is deflated by recompression at depth. Diamond et al.[7] tested the Shelton Fish Descender[9] (Shelton Products, Newark, CA) and showed that the use of that bottom-release device did not improve survival over the use of a treatment in which fish were vented and released at the surface. Another device that releases fish at a preset depth through a pressure-sensitive clamp was tested in a different study that showed that fish released through the use of that device are more likely to survive than fish vented and released at the surface (Stunz and Curtis[10]). At this time, it is difficult to discern if the differences between these 2 experiments were due to the gear used or some other effect, such as low sample sizes. The significant interaction term from the results of these studies indicates that release devices may be useful in reducing mortality in lieu of venting. More studies are needed

[9] Mention of trade names or commercial companies is for identification purposes only and does not imply endorsement by the National Marine Fisheries Service, NOAA.

[10] Stunz G. W., and J. Curtis. 2012. Examining delayed mortality in barotrauma afflicted red snapper using acoustic telemetry and hyperbaric experimentation. SEDAR31-DW21, 15 p. [Available from http://www.sefsc.noaa.gov/sedar/.]

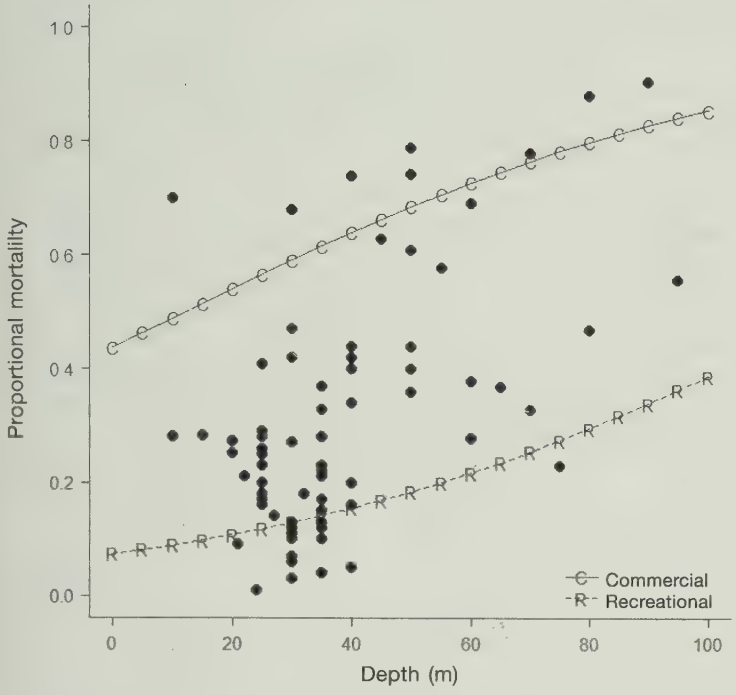

Figure 6

Predicted relationship between depth and release mortality (proportion) of red snapper (*Lutjanus campechanus*) in the Gulf of Mexico derived from the meta-analytical model for the commercial and recreational fishing sectors. Results indicate that mortality rates are significantly higher in the commercial fishing sector than in the recreational sector.

to determine whether there is some potential for bottom-release devices to enhance postrelease survival or whether the effects of barotrauma cannot be reversed as was suggested by Wilde (2009).

Season also was a significant factor that affected release mortality and, because season is a rough proxy for water temperature, the data indicate a positive relationship between water temperature and release mortality of red snapper. Impairment-scaling metrics that evaluate sublethal effects of CAR fishing, such as reduction of reflex responses, also show similar relationships with water temperature (Diamond and Campbell, 2009; Campbell et al., 2010a, 2010b). Furthermore, impairment-scaling metrics were linked to increased immediate estimates in at least 2 of those studies. Tagging data show that the lowest returns have been for fish tagged during summer and the highest recapture rates have been for fish tagged during the winter (Diamond et al.[7]; Sauls[8]), although tag-and-recapture studies are heavily influenced by the timing of the primary effort in a fishery (i.e., winter fishing effort is low and may result in fewer recaptures during that time).

Finally, in 3 projects that required field collections before laboratory investigations could begin, red snapper were unable to be kept alive during collection or transport back to a laboratory during summer months (Parker[5]; Burns et al.[6]; Campbell et al., 2010a). Most investigations included in our meta-analysis had vaguely defined seasonal classifications, and other studies reported the months in which sampling took place. A single study reported water temperatures and thermocline strength. Vague seasonal classifications of sampling time frames complicate information from transitional seasons, such as fall, because September water temperatures in the GOM often are more reflective of summer conditions. Evidence of unexplained residual heterogeneity in the mixed-effects model might be associated with insufficient treatment of these thermal components; therefore, future studies should focus attention on this relationship.

Another common problem found in this meta-analysis was that the acoustic-tagging and caging studies typically had limited sample sizes. With one exception, the caging studies evaluated in this meta-analysis had depth-specific sample sizes of less than 56 fish (Parker[5]; Gitschlag and Renaud, 1994; Render and Wilson, 1994; Diamond and Campbell, 2009). The acoustic-tagging study available for inclusion split 44 fish between summer and winter sampling efforts (Diamond et al.[7]). Low sample sizes can lead to poor estimation of effects because proportions are unstable at low sample sizes. Another issue is that, because of the ease of obtaining surface-release estimates, those studies greatly outnumbered other types of studies in this meta-analysis. Furthermore, the sample-size weighting scheme in this meta-analysis lends more weight to experiments with large samples, and that weighting potentially biases outcomes toward surface-release studies.

Acoustic or satellite tags give the ideal level of information, but until the expense of tags and required monitoring systems is reduced, those studies will be hampered by small sample sizes and poor statistical power to estimate mortality. Another complicating factor in acoustic-tagging and caging studies is that handling and abrasion can act to increase release mortality rates (Jarvis and Lowe, 2008; Hannah et al., 2012). Recent development of a novel caging system to evaluate survival was effective in reduction of mortality due to abrasion for several rockfish species (Hannah et al., 2012). Similarly, the use of an external acoustic tag to evaluate rockfish survival has shown promise in reducing handling times and may help to increase sample sizes in acoustic-tagging studies (Hyde[11]). Methods

[11]Hyde, J. 2013. Personal commun. NOAA Southwest Fisheries Science Center, La Jolla, CA 92037.

Table 4

Predicted estimates of release mortality from a meta-analytical model of red snapper (*Lutjanus campechanus*) in the Gulf of Mexico, by depth (0–100 m), for the average model run (Average) of equally weighted coefficients, season (winter, spring, summer, and fall), and fishing sector (Comm.=commercial and Rec.=recreational). Predicted estimates were derived with the coefficients presented in Table 2 and shown in graph format in Figures 3–6).

Depth	Average	Winter	Spring	Fall	Summer	Annual	Comm.	Rec.
0	0.199	0.131	0.159	0.225	0.289	0.220	0.437	0.074
5	0.216	0.144	0.173	0.244	0.311	0.239	0.463	0.081
10	0.235	0.157	0.189	0.264	0.334	0.258	0.489	0.089
15	0.254	0.171	0.205	0.285	0.358	0.278	0.515	0.098
20	0.274	0.187	0.223	0.306	0.382	0.300	0.541	0.108
25	0.295	0.203	0.241	0.329	0.407	0.322	0.567	0.118
30	0.318	0.221	0.261	0.352	0.432	0.345	0.592	0.130
35	0.341	0.239	0.281	0.377	0.458	0.369	0.617	0.142
40	0.364	0.258	0.303	0.401	0.484	0.394	0.642	0.155
45	0.389	0.279	0.325	0.427	0.510	0.419	0.665	0.169
50	0.414	0.300	0.349	0.452	0.536	0.445	0.688	0.185
55	0.439	0.323	0.373	0.478	0.562	0.471	0.710	0.201
60	0.465	0.346	0.397	0.504	0.588	0.497	0.731	0.218
65	0.491	0.370	0.423	0.530	0.613	0.523	0.751	0.236
70	0.517	0.395	0.448	0.556	0.637	0.549	0.770	0.256
75	0.543	0.420	0.474	0.582	0.661	0.574	0.788	0.276
80	0.569	0.445	0.500	0.607	0.684	0.600	0.805	0.297
85	0.595	0.471	0.526	0.632	0.706	0.624	0.821	0.320
90	0.619	0.497	0.552	0.656	0.727	0.649	0.836	0.343
95	0.644	0.523	0.578	0.679	0.747	0.672	0.849	0.367
100	0.667	0.549	0.603	0.701	0.766	0.695	0.862	0.391

that reduce the effects of handling and that increase sample sizes are encouraged regardless of the study type chosen.

Because of the need for estimates derived from fishery-dependent surveys that accurately reflect fishing practices, passive-tagging studies might be the best method available, but they still have problems associated with their use. Passive-tagging surveys require large numbers of fish to evaluate survival because recapture rates are typically low in the red snapper fishery (<10%). Furthermore, only 1 of the passive-tagging studies evaluated here produced estimates through the use of a tag-and-recapture model (Sauls[8]), although the other 2 studies made use of surface-release methods (Patterson et al., 2001; Patterson[4]). Recent modeling efforts in other fisheries have shown promise in the use of recapture and impairment scaling data to calculate relative survival from risk-ratio models (Sauls[8]; Sauls, 2014). Continued development of tag-and-recapture models would be useful because 1) such models potentially avoid the biases associated with other estimation methods, 2) there is an abundance of tagging data available, and 3) studies can be designed to directly observe the fishery as it is prosecuted.

Methods for calculating or scaling the level of impairment of fish caused by effects of CAR fishing have proven to be useful for the estimation of release mortality for many species, including walleye pollock (*Gadus chalcogrammus*, Gadidae), coho salmon (*Oncorhynchus kisutch*, Salmonidae), sablefish (*Anoplopoma fimbria*, Anoplopomatidae), northern rock sole (*Lepidopsetta polyxystra*, Pleuronectidae), lingcod (*Ophiodon elongatus*, Hexagrammidae), Pacific halibut (*Hippoglossus stenolepis*, Pleuronectidae), and red snapper (Davis et al., 2001; Davis and Ottmar, 2006; Davis, 2007; Campbell et al., 2010a, 2010b). The impairment scaling metric for the barotrauma reflex showed a positive logistic relationship between impairment level and immediate estimates of release mortality in the recreational red snapper fishery for both surface-release and caging studies (Campbell et al., 2010a; Diamond and Campbell, 2009). Because impairment-scaling studies have shown significant relationships with release mortality in both the surface-release and caging studies, these techniques may prove to be useful in tag-and-recapture models.

We did not find a significant reduction in mortality by hook type, which was surprising given that the regulation requiring circle hooks was thought to be effective in reduction of discard mortality by decreasing the frequency of gut hooking. However, any effects of circle hooks on discard mortality may have been con-

founded in this study with fishing sector because the only commercial study that was available exclusively used circle hooks and many recreational studies used a mixture of hooks and a variety of venting practices. Furthermore, few studies reported hook sizes, and it is likely that the incidence of gut hooking, which can significantly increase release mortality, is related to hook size because mouth gape limits the effectiveness of large hooks. Therefore, our ability to estimate an effect of circle hooks separate from fishing sector, study type, and season may have been diminished. We do not think that circle hooks lack positive benefits; we simply may not have been able to detect them from the available studies, and we know little about the potential interactions of other variables with hook size. Nonetheless, substantial documentation indicates that there are positive benefits associated with circle hooks (Cooke and Suski, 2004).

Conclusions

There have been significant improvements in understanding release mortality in general and particularly in the red snapper fishery. Despite the significant efforts of many researchers, fundamental biases still persist in the various approaches. Estimates from surface-release studies do not address long-term effects of barotrauma, do not account for predation, and rely on submergence ability as a proxy to calculate mortality rates. Delayed estimates have been hampered by small sample sizes, cost prohibitive designs, excessive handling, and failure to duplicate normal conditions when releasing fish. Venting results were contingent upon the timing aspect specific to the various methods being used (i.e., delayed and immediate timing of estimates).

A focus on increasing sample sizes in acoustic-tagging surveys and continued improvement of tag-and-recapture models would be useful. Passive- and acoustic-tagging appear to offer good solutions because they can measure both immediate and delayed components and fish handling biases can be minimized, particularly as technology improves and costs are brought down. Experiments in which impairment scaling is estimated and that include both immediate and delayed estimates also would be valuable for the further development of those relationships within models and potential adjustment of historical estimates. Future surveys should include some, if not all, of the following properties: quarterly sampling, appropriate range of depths, water temperature and thermocline data rather than seasonal categorization, tag-and-recapture modeling, and measurement of barotrauma and reflex responses.

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In cold blood: evidence of Pacific sleeper shark (*Somniosus pacificus*) predation on Steller sea lions (*Eumetopias jubatus*) in the Gulf of Alaska

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Abstract—Temperature data received post mortem in 2008–13 from 15 of 36 juvenile Steller sea lions (*Eumetopias jubatus*) that had been surgically implanted in 2005–11 with dual life history transmitters (LHX tags) indicated that all 15 animals died by predation. In 3 of those 15 cases, at least 1 of the 2 LHX tags was ingested by a cold-blooded predator, and those tags recorded, immediately after the sea lion's death, temperatures that corresponded to deepwater values. These tags were regurgitated or passed 5–11 days later by predators. Once they sensed light and air, the tags commenced transmissions as they floated at the ocean surface, reporting temperatures that corresponded to regional sea-surface estimates. The circumstances related to the tag in a fourth case were ambiguous. In the remaining 11 cases, tags sensed light and air immediately after the sea lion's death and reported temperatures that corresponded to estimates of regional sea-surface temperatures. In these 11 cases, circumstances did not allow for inferences on the species of predator. Among reported poikilotherm predators of Steller sea lions, only the Pacific sleeper shark (*Somniosus pacificus*) is known to have body core temperatures that are near ambient. The data from this study indicate that Pacific sleeper sharks need to be considered as a possible source of mortality of juvenile Steller sea lions in the region of the Gulf of Alaska.

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The western distinct population segment (WDPS) of the Steller sea lion (*Eumetopias jubatus*) (west of 144°W) has declined to approximately 20% of the levels encountered before 1975 (National Research Council, 2003), and declines were at or above 5% per year in some areas. Recent data indicate trends of slightly increasing population overall and continuing steep declines for the western Aleutian Islands from 2000 to 2012 (Fritz et al., 2013). Limited trend data indicate stable or slightly increasing levels for the Prince William Sound–Kenai Fjords area of the Gulf of Alaska (Fritz et al., 2013).

Multiple factors have been posited as driving past and present population trends of the Steller sea lion (National Research Council, 2003). York (1994) hypothesized reduced juvenile survival in the Gulf of Alaska region during the height of the decline. Holmes et al. (2007) hypothesized that juvenile survival rates for the period of 1997–2004 in the same region had recovered but that birth rate had steadily declined. Specific proximate drivers of the hypothesized reduced juvenile survival or reduced birth rates remain unknown. Suggested causes include nutritional stress through competition with commercial fisheries (National Research Council, 2003), the "inadequate food/ocean climate hypothesis" (e.g., Trites et al., 2007), and the "predation hypothesis" (e.g., Williams et al., 2004). However, to date no direct test of any of these hypotheses has been published in peer-reviewed literature for any region of the WDPS.

Direct observations (Ford et al., 1998; Maniscalco et al., 2007), physical evidence (Heise et al., 2003), and telemetered data (Horning and Mellish, 2009, 2012) reveal the occurrence of predation on Steller sea lions. From a review of research conducted through the 1990s, the National Research Council (2003) concluded that the recovery of the WDPS was more likely limited by predation than by resource-driven effects. Reported predators of Steller sea lions include not only the transient ecotype of the killer whale (*Orcinus orca*) (Ford et al., 1998) but also the white shark (*Carcharodon carcharias*), the salmon shark (*Lamna ditropis*), and the Pacific sleeper shark (*Somniosus pacificus*) (Loughlin and York, 2000). However, the accurate detection and quantification of sea lion depredation at sea has been labeled as "empirically intractable" (Williams et al., 2004).

To address this constraint, we specifically developed the life history transmitter (LHX tag; Wildlife Computers[1], Redmond, WA; described in Horning and Hill, 2005) to detect and determine causes of Steller sea lion mortality irrespective of time and location of mortality, or age of host. LHX tags were designed to remain implanted through the entire life of the host (Horning et al., 2008). Surgical implantation allows multiyear monitoring beyond the annual molt that limits the use of externally attached devices. LHX tags do not transmit while inside the host body, greatly extending battery life beyond 10 years. Tags liberated from decomposing, dismembered, or digested bodies send transmissions to the Argos telemetry system (CLS America Inc., Lantham, MD) aboard NOAA satellites with global coverage (Soma and Tsutsumi, 1986). LHX tags, therefore, provide postmortem data on time and date, location, and causes of mortality with spatially and temporally unlimited resight effort (Horning and Mellish, 2009). Here, we provide indirect evidence (from LHX tag data) of predation by Pacific sleeper sharks on juvenile Steller sea lions.

Materials and methods

This study was carried out in strict compliance with all applicable animal care and use guidelines under the U.S. Animal Welfare Act and was approved as required under the U.S. Marine Mammal Protection Act and the U.S. Endangered Species Act by the National Marine Fisheries Service (permit numbers 1034-1685, 1034-1887, 881-1890, 881-1668, 14335, and 14336) and by the Institutional Animal Care and Use Committees of the Alaska Sea Life Center (permit numbers 02-015, 03-007, 05-002, 06-001, 08-005, and R10-09-04) and Texas A&M University (permit numbers 2003-181, 2005-170, and 2006-37). All surgeries were performed under aseptic conditions and under full anesthesia administered by inhalant gas, and all efforts were completed in a way meant to minimize pain and suffering.

Life history transmitters

We used satellite-linked LHX tags (Horning and Hill, 2005) to determine survival rates and infer causes of mortality in juvenile Steller sea lions in the Gulf of Alaska. LHX tags are intraperitoneally implanted archival transmitters that monitor sensor data through the life of host animals. Select recorded data are transmitted (uplinked) post mortem to the Argos system aboard NOAA satellites, after the positively buoyant tags are liberated from a decomposing, dismembered, or digested carcass. LHX tags provide 48 h of temperature data across detected mortality events. In 30-min increments, 96 temperature values are recorded with an accuracy better than 0.1°C and with a nonlinear resolution from 0.2°C (at 0°C) to 0.25°C (at 40°C), for 48 ante mortem and 48 postmortem values. Mortality is detected when temperature drops below the preset threshold of 24°C. In addition to a temperature sensor, LHX tags incorporate a radiometric irradiance sensor and a proprietary radio-frequency immersion sensor (see Horning and Hill, 2005) that allows the classification of the medium surrounding the antenna portion of a tag as tissue, saltwater, or air.

Tags begin to transmit after they sense surrounding air or sufficient ambient light. Thereafter, current temperature (hereafter, called *uplink temperature*) and irradiance are included in each transmission. Additional diagnostic sensor data transmitted by the tags allow the classification of ambient light conditions from time of death to onset of transmissions. Light conditions are classified into the categories of complete darkness or light exceeding a preset irradiance threshold of 2.9×10^{-8} Wcm^{-2} (for λ=527–583 nm). This threshold corresponds with the approximate penetration depth of radiant energy in the ocean that ranges from 0 m for a starlit night to 25 m for a night with a full moon, 100 m for a dark, cloudy day, and 200 m for a bright day, as estimated from the optical properties of seawater reported for the Alaska Gyre (Sasaki et al., 2001). Tags perform a daily test of the irradiance sensor through a built-in LED light source, and any faults are reported. For short-term diagnostic monitoring of postoperative recovery, LHX tags were programmed to transmit tag temperatures every 15–60 min for periods ranging up to 1–2 weeks after implantation. When the animals were not submerged in the saltwater pools of the recovery facility, these data were received with a nearby (<20 m) handheld uplink receiver.

The Argos system provides approximate locations of uplinking tags with accuracy indicated within a range from 0.49 km to 4.18 km (Soma and Tsutsumi, 1986; Costa et al., 2010). However, LHX tags commence transmissions after extrusion only at local noon. This timing may result in the estimates of the first position occurring up to 24 h post mortem for immediate extrusion events or after longer periods for delayed extrusions. We previously have estimated event location accuracy at approximately 10 km (Horning and Mellish, 2009).

Inferring causa mortis from temperatures

The temperature, irradiance, and immersion data allow the classification of detected mortality events as 1) death at sea by massive trauma likely due to predation or as 2) nonpredation death (Horning and Mellish, 2009). The normal abdominal temperature of healthy pinnipeds—although not well studied—is thought to range from 36°C to 38°C (Whittow, 1987). Antemortem temperature patterns may reveal hypothermia or pyrexia (fever), the former indicative of chronic energetic

[1] Mention of trade names or commercial companies is for identification purposes only and does not imply endorsement by the National Marine Fisheries Service, NOAA.

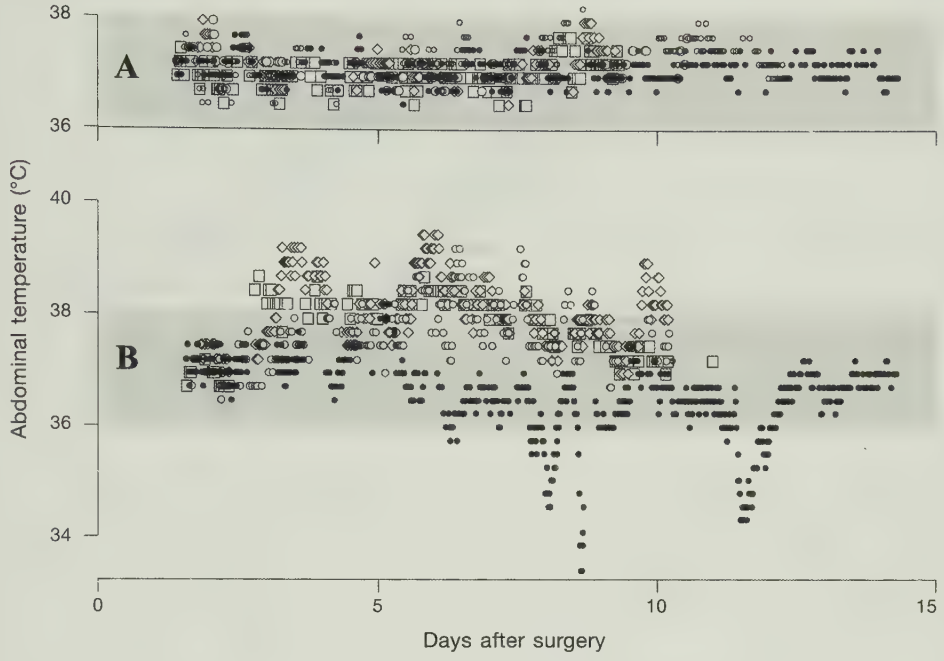

Figure 1

Postoperative abdominal temperatures transmitted from life history transmitters (LHX tags) implanted in Steller sea lions (*Eumetopias jubatus*). (**A**) Temperatures in 5 animals that did not exhibit inflammation or other apparent health challenges. The animals were held in temporary captivity for up to 1 month at the Alaska Sea Life Center in Seward, Alaska, between 2005 and 2011. (**B**) Elevated temperatures in 3 animals that exhibited postoperative pyrexia (>38.2°C) in response to localized inflammation that surrounded the surgical incision, and 1 animal that exhibited postoperative hypothermia (<36.0°C) associated with inappetence and lethargy. The gray shaded areas indicate the normal range of abdominal temperatures for healthy sea lions.

imbalance and starvation (Rosen et al., 2007), the latter indicative of inflammatory responses to immune challenges (Stoskopf, 2005).

During our postoperative monitoring of captive sea lions, most sea lions exhibited body core temperatures between 36.0°C and 38.2°C (Fig. 1A). However, several animals that were experiencing immunological challenges in the form of temporary, localized inflammations around the sutures at the incision site exhibited repeated abdominal temperatures above 38.2°C and up to 39.4°C for multiple days (Fig. 1B). Inflammation was evident in the form of visible discharge accompanied with localized swelling and localized elevation of surface temperatures determined with infrared thermography. One animal that was experiencing depressed appetence and lethargy exhibited reduced abdominal temperatures repeatedly over several days (Fig. 1B). The temperatures of all animals returned within the normal range of 36.0–38.2°C before their release. Exertion hyperthermia may temporarily elevate temperatures to 39°C, but such events are limited to <1 h. Therefore, sustained (longer than several hours) antemortem temperatures outside of the range from 36°C to 38°C range should be indicative of compromised health conditions, such as starvation or disease.

Immediately after the death of warm-blooded animals, intact bodies will gradually cool over a period of several hours at rates proportional to initial body temperature and mass, insulation, and type, temperature, and flow rate of the surrounding medium (Marshall and Hoare, 1962). Models of algor mortis (postmortem cooling) have been applied in human forensic pathology to estimate time of death when body mass is known (Henssge, 1995). When time of death and cooling rates are known, the same models can be solved for mass. We previously have parameterized an algor mortis model for sea lions (Horning and Mellish, 2009). This sea lion model reasonably predicts cooling of sea lion carcasses in air and water (Fig. 2, D and E) and allows for estimation of mass at time of death. If implanted LHX tags remain embedded in intact bodies after death, then they should record gradual cooling while in darkness and surrounded by tissue. Such temperature data should produce cooling curves similar to those shown in

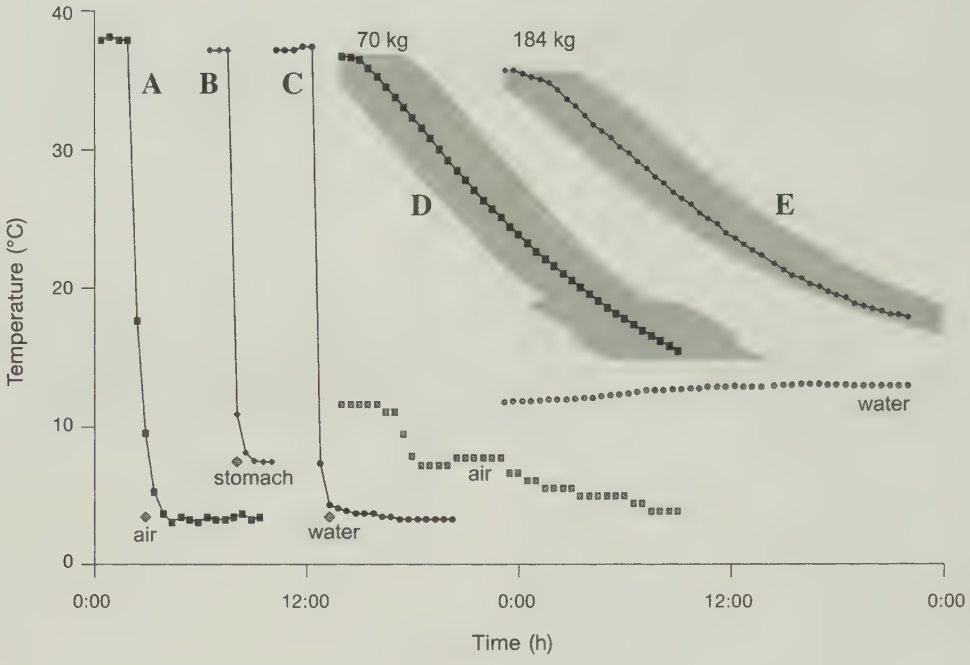

Figure 2

Temperatures recorded by life history transmitters (LHX tags) during different cooling experiments in the laboratory. Temperatures were recorded in 30-min increments by LHX tags equilibrated at approximately 36–38°C and subsequently transferred to different media, such as air, water, and stomach tissue. Temperatures of the transfer media are indicated by gray symbols. (**A**) LHX tag transfer from water bath to air at 3.5°C. (**B**) LHX tag transfer from water bath into gastric tissue at 7.5°C. (**C**) LHX tag transfer from water bath into water at 3.5°C. (**D**) LHX tag abdominally implanted into a 70-kg California sea lion (*Zalophus californianus*) immediately post mortem at the Alaska Sea Life Center in Seward, Alaska, and the carcass then cooling in ambient air at 4–12°C (gray squares). (**E**) LHX tag abdominally implanted into a 184-kg California sea lion at The Marine Mammal Center in Sausalito, California, immediately post mortem, and the carcass cooling in ambient water at 12–13°C (gray circles). Shaded areas for D, E represent ranges of 95% confidence intervals from the outputs of the algor mortis model (for details, see the *Materials and methods* section). Measurements shown for A, B, and C were data collected during laboratory experiments conducted at Oregon State University in Newport, Oregon, in 2005. The sea lion carcasses were made available by regional stranding networks in Alaska (2008) and California (2007). The sea lions were euthanized before insertion of LHX tags for reasons unrelated to this project.

Figure 2, D and E. Tags that remain embedded in parts of dismembered bodies also should record temperatures that indicate gradual cooling but at rates considerably faster than the cooling rates for intact bodies (Horning and Mellish, 2009). Alternatively, the temperatures of tags that are fully liberated from dismembered bodies should quickly adjust to temperatures of the surrounding medium, as illustrated in Figure 2, A–C.

The above listed considerations indicate 4 possible causae mortis scenarios (see Horning and Mellish, 2009).

Type I: Very rapid drops in postmortem temperature (as shown in Figure 2, A and C) that are associated with immediate sensing of ambient light or air must be the result of direct tag extrusion. Such a sequence of events, when combined with the immediate onset of transmissions, indicates traumatic death by dismemberment leading to immediate tag extrusion, most likely due to predation. A type-I scenario also could be caused by scavenging if this process releases the tags from the body and if it occurred within 60 min of death (depending on the mass of the sea lion; otherwise, the onset of gradual cooling would be detectable). In this case, scavenging would have been preceded immediately by death due to other causes, and any deaths caused by disease or starvation additionally should be associated with antemortem temperatures outside of the normal range of 36.0–38.2°C.

Type II: A gradual temperature drop with substantially delayed sensing of light or air and delayed onset

of transmissions, as shown in Figure 2, D and E, indicates that the tag remained in the carcass as it cooled. This scenario suggests a nontraumatic death resulting from disease, starvation, or drowning due to entanglement. Events caused by disease or starvation additionally should be associated with antemortem temperatures outside of the normal range of 36.0–38.2°C.

Type III: Gradual but quicker than predicted cooling with delayed sensing of light and air may indicate traumatic predator attacks and partial dismemberment even if tags remain embedded in tissue.

Type IV: Very rapid drops in postmortem temperatures, as shown in Figure 2B, without immediate sensing of light or air indicate dismemberment and tag ingestion by a cold-blooded predator. Ingestion by a warm-blooded predator would result in no discernible changes in temperature and light until expulsion from the predator's digestive tract. A type-IV scenario also could be caused by scavenging if it occurred and if the tags were ingested within 60 min post mortem (otherwise, the onset of gradual cooling would be detectable). In this case, scavenging would have been preceded immediately by death due to other causes. Any deaths caused by disease or starvation additionally should be associated with antemortem temperatures outside of the normal range of 36.0–38.2°C.

Tag deployments and controls

We implanted LHX tags into 36 juvenile Steller sea lions captured in Prince William Sound, Alaska, from 2005 to 2011 (Horning and Mellish, 2012). Dual LHX tags were used in all but the first 2 animals to increase and determine probability of detection of a mortality event (the first 2 animals received only single implants). LHX tags were implanted intraperitoneally while the sea lions were under gas anesthesia and according to standard aseptic surgical techniques (Horning et al., 2008). All animals were released in Resurrection Bay in the Kenai Fjords region, at ages of 14–26 months and at a mean mass of 128.6 kg (standard deviation [SD] 28.6; range: 73–198 kg). In addition to live animal deployments, we conducted 10 simulation tests by implanting dual LHX tags in sea lion carcasses obtained from regional stranding networks and subsequently deposited ashore (n=5) or at sea (n=5) in California and Oregon.

Previously published control studies with animals monitored during temporary captivity (21–71 d) and tracked for a mean postrelease period of 86 d (SD 55; range: 10–242 d; n=35) for implanted animals and of 76 d (SD 54; range: 11–194; n=30) for nonimplanted animals—with all animals having conventional satellite transmitters externally attached after their release (Wildlife Computers SDRT-16 and SPLASH-5 tags)—confirmed that 1) implant surgery results in mild to moderate wound healing responses and temporary elevation of white cell counts and haptoglobin concentrations, with full physiological recovery within 45 d following surgery; 2) tags and surgery result in zero mortality to 45 d; 3) postrelease foraging and ranging behavior does not differ between implanted and nonimplanted animals or between temporarily captive and free-ranging animals; and 4) the cumulative survival of animals over the ages of 14–60 months was 0.415 (95% confidence intervals [CI]=0.26–0.63), compared with 0.413 (95% CI=0.27–0.55) for mark-resight studies based on hot iron branding conducted by the National Marine Fisheries Service, providing no evidence of any effects of LHX tags or implant surgery on survival to the age of 5 years (Mellish et al., 2006, 2007; Thomton et al., 2008; Petrauskas et al., 2008; Walker et al., 2009; Horning and Mellish, 2012).

Received data and environmental data

We estimated probability of event detection from the ratio of dual to single tag returns. We first estimated uplink failures (the combination of technical tag failures and transmissions from a functional tag that did not reach any satellite because of tag exposure constraints) as

$$P_{\text{fail}} = C_{\text{single}} / (C_{\text{single}} + 2C_{\text{dual}}),$$

where C_{single} = the count of single returns; and
C_{dual} = the count of dual returns.

Then, a correction factor F was derived as $F = 1 / (1-P_{\text{fail}}^2)$, and the corrected number of mortality events E_{corr} was calculated as $E_{\text{corr}} = F\,(C_{\text{single}} + C_{\text{dual}})$. From P_{fail}, the probability of event detection P_{detect} in turn was derived as $P_{\text{detect}} = 1-P_{\text{fail}}^2 = 1/F$. Ranges containing 95% of the likely variance for the estimate of P_{fail} were derived from the cumulative distribution function of a Monte Carlo simulation (>2500 iterations) of randomly assigned individual tag failures for $0 < P_{\text{fail-simulated}} < 1$ yielding P_{fail} not exceeding the observed value without increasing E_{corr} integer counts. The range of 95% CI for P_{fail} in turn yielded confidence intervals for F and E_{corr}.

Sea-surface temperature (SST) composite data derived from the NOAA Geostationary Operational Environmental Satellites (GOES) and Polar-orbiting Environmental Satellites (POES) were obtained for specific dates from the Comprehensive Large Array-data Stewardship System (CLASS) (SST50 or SST100 products available from http://www.nsof.class.noaa.gov/saa/products/welcome, accessed June 2013). In situ SST data for specific dates were obtained from NOAA's National Data Buoy Center (available from http://www.ndbc.noaa.gov/, accessed June 2013). Long-term averages of water column temperature profiles from 1970 to 2012 for the GAK1 location, at 59°50.7′N, 149°28.0′W, near the Kenai Fjords region of the northern Gulf of Alaska were obtained from a time-series data set of the University of Alaska's Institute of Marine Science (available from http://www.ims.uaf.edu/gak1/, accessed June 2013). Temperature estimates for deeper waters

Table 1

Animal and event details for 15 mortalities, for which complete data sets were available, detected in juvenile Steller sea lions (*Eumetopias jubatus*) in the Gulf of Alaska between 2005 and 2013. Two values within a cell represent data returns from dual life history transmitters (LHX tags) implanted in sea lions, and, in the case of single values, only data from one tag was received. Mass at death was predicted from mean growth rates for age class and sex (Winship et al.[1]) applied to mass at release. Postmortem temperature (T) is the mean of values recorded from time of death for 24 h or to onset of data transmissions from tags. In cases for which a temperature range for 24 h post mortem is not provided, tags began transmissions in less than 24 h. SST=sea-surface temperature.

Animal no.	52	57	51	44	54	63	35	64	46	47	58	59	32	33	62
Month of death	Aug	Sep	Oct	Nov	May	Dec	May	Dec	Jan	Jan	Feb	Jan	Feb	Feb	Mar
Age at death (y)	4.1	2.3	1.4	2.4	1.9	1.5	1.9	1.5	2.6	1.6	1.7	2.6	1.7	1.7	2.7
Predicted mass at death (kg)	243	137	110	161	96	121	163	130	171	110	116	152	158	144	148
Days to transmit	11	0.1	0.6	1	0.2	1	0.4	5	0.4	0.1	1	0.2	16	0.9	0.6
T post mortem (°C)	5.53	0.1	0.6	1	6.77	1	0.4	9	0.4	0.1	1	0.2	4.51	0.9	0.6
		10.4	9.81	8.58		5.20	5.07	7.55	4.96	4.75	3.96	3.37		3.27	3.60
		10.5	9.66	8.41		7.02	4.56	7.54	4.79	4.54	4.04	3.42		3.16	3.51
T range 24 h post mortem (°C)	0.0	–	–	2.2	–	1.8	–	0.0	–	-	1.4	–			
				2.4		0.2		0.0			0.8				
Regional SST (°C)	11.6	10.6	9.6	8.2	6.9	5.1	5.0	5.0	4.9	4.7	4.0	3.7	3.7	3.4	4.1
SST source[2]	3, 7	4	3, 6	4, 11	3, 6	8, 13	3, 9	8, 13	3, 10	3, 5	4, 8	7, 13	12, 13	13	6, 13

[1] Winship, A. J., A. W. Trites, and D. G. Calkins. 2001. Growth in body size of the Steller sea lion (*Eumetopias jubatus*). J. Mammal. 82:500–519.

[2] Values from multiple sources were averaged (see *Materials and methods* section): [3] Comprehensive Large Array-data Stewardship System; NOAA Data Buoy No. or identifier: [4] 46060, [5] 46061, [6] 46076, [7] 46080, [8] 46081, [9] NKTA2, [10] SNDA2, and [11] VDZA2; [12] GAK1 station of the Institute of Marine Science, University of Alaska Fairbanks; [13] first uplink from LHX tag.

in Prince William Sound were averaged from values reported for locations in the Whale Bay, Eaglek Bay, and Zaikof Bay by Gay and Vaughan (2001).

Results

We detected 17 mortality events between 22 November 2005 and 15 June 2013, over 40,620 cumulative exposure days in 36 implanted animals (28,202 days over the age range of 14–60 months). All 17 events were from animals with dual implants. Thirteen events yielded dual returns, and 4 events yielded single returns. Therefore, from the 34 tags implanted in the 17 animals that died, we recovered 27 full and 3 partial data sets; 4 tags returned no data. We recovered 18 full data sets from 20 tags in 10 carcass simulations; 2 tags yielded no data. Tags from carcasses uplinked on average within 32 days of deposition (SD 14.2; range: 14–65 d; n=18). From the ratio of dual (n=21) to single (n=6) data returns from the 52 tags in 27 events, we estimated event detection probability at 98.4% (CI=95.0–100.0). This result indicates that we most likely did not miss any occurrences of mortality within our sample of 36 animals implanted with transmitters.

Of the 17 detected events, 2 yielded insufficient data to allow for inferences on causes of mortality. Complete temperature and diagnostic data sets from at least 1 tag per animal were produced from each of 15 events (Table 1). Antemortem temperatures in all of the15 detected mortality events with data were within normal ranges for healthy animals (36.0–38.2°C), and there as no evidence of pyrexia or hypothermia (Figs. 3–5). All of these 15 events occurred at sea and were classified as predation, as detailed later. The combined probability distribution of single to dual tag returns of 2+13 for the 15 predation events and 2+8 for the 10 carcass tests that constitute simulated nontraumatic deaths gave an odds ratio of 1.625, indicating no differences in detection probabilities between predation events and simulated nontraumatic events (Fisher's exact probability test: $P_{(2,1)}$=1.0; Fisher, 1922).

Of the 15 detected predation events, 11 events fell into a type-I scenario. In these events, temperatures dropped precipitously, with a mean peak ΔT of –0.81°C min^{-1} (SD 0.1), to ambient values that corresponded to estimates of regional SSTs (Fig. 3; Table 1). This rate of

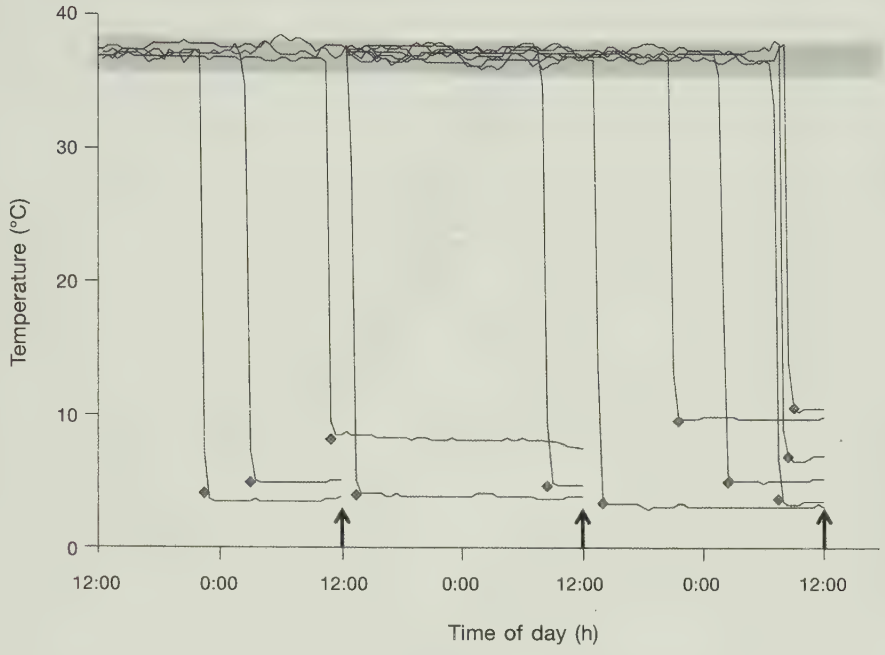

Figure 3

Antemortem and postmortem temperatures recorded by life history transmitters (LHX tags) for 11 mortality events detected in juvenile Steller sea lions (*Eumetopias jubatus*) with rapid onset of transmissions. Temperatures were recorded by LHX tags in 30-min increments and subsequently transmitted through the Argos satellite system. All events occurred in the Gulf of Alaska between 2008 and 2013 (for further details, see Table 1 and the *Materials and methods* section). Data from only one tag are shown for each animal. If data from both tags were available, then the data patterns from one tag that are not shown here did match the patterns of the other tag shown here. Because these 11 events occurred on different dates, data from one tag from each event were arbitrarily assigned to one of 3 sections of this graph solely for clarity. Gray diamonds indicate the regional estimates of sea-surface temperatures (for sources, see Table 1). All tags sensed light or air by or before they began to transmit at the end of each temperature record; arrows indicate the end of records and onset of transmissions for each tag. The gray shaded area at the top of this graph indicates the normal range of abdominal temperatures for healthy sea lions (Fig. 1).

decline in peak temperature is consistent with the rate observed in laboratory simulations (n=15) of tag transfers to water: 0.88°C min^{-1} (SD 0.14; Fig. 2C; Horning and Mellish, 2009). In all 11 cases, tags uplinked at the first opportunity after each had sensed ambient light, air, or both, indicating immediate tag extrusion at sea. This notion was confirmed by the locations of initial uplinks at sea. In all 11 events, the recorded postmortem temperatures coincided with regional SST estimates (Fig. 3; Table 1).

One type-III event was observed. This event yielded only a single complete data set with temperatures that gradually declined (peak ΔT=–0.16°C min^{-1}) over 18+ h to 4.51°C, close to the regional SST of 3.98°C (Fig. 4; Table 1). Transmissions began 16 d post mortem. The algor mortis model yielded a mass estimate of only 24 kg, significantly below the mass of the animal at release (146 kg; model output shown in Fig. 4) and the estimated mass at the age of death (158 kg), and indicating partial dismemberment (Horning and Mellish, 2009).

Of the remaining 3 events, 2 events fell into a type-IV scenario. In the first event (TJ52), only a single transmitter uplinked. This tag recorded a precipitous temperature drop (peak ΔT = –0.98°C min^{-1}) to 5.53°C, but the tag did not sense light or air and did not transmit until 11 d post mortem (Table 1; Fig. 5). Multiple uplink temperatures of 11.98°C corresponded to the regional SST estimate of 11.90°C, more than double the recorded postmortem temperatures.

In the second event (TJ64), both tags sensed precipitous drops (peak ΔT=–0.93°C min^{-1}) to a temperature

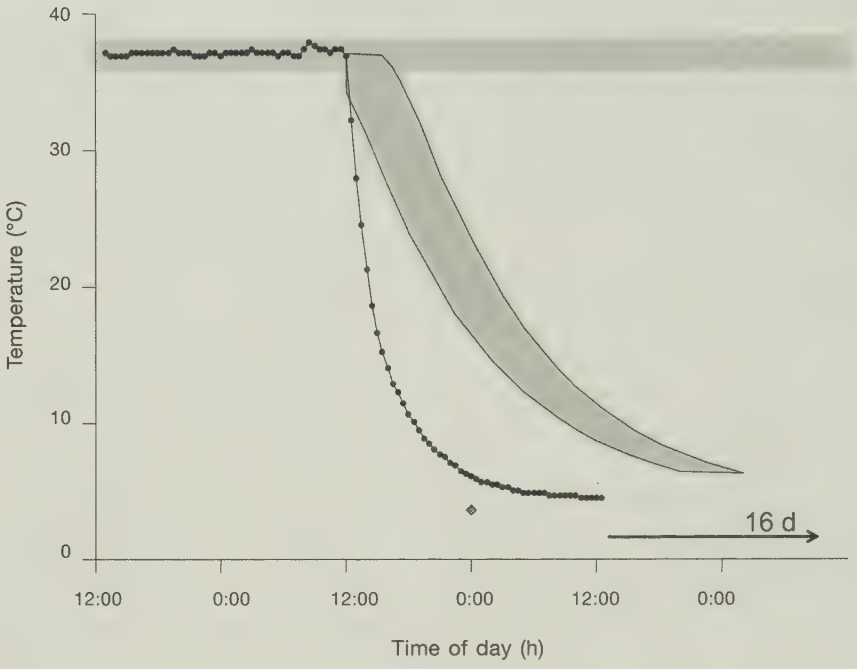

Figure 4

Postmortem temperatures indicating gradual cooling recorded for the mortality of one Steller sea lion (*Eumetopias jubatus*) by a life history transmitter (LHX tag) with delayed onset of transmission. Temperatures were recorded in 30-min increments by the single, abdominally implanted LHX tag. The event occurred in the Gulf of Alaska in 2008. The gray shaded area represents the 95% confidence interval from the output of the algor mortis model for a 146-kg animal (for details, see the *Materials and methods* section). Actual cooling rates were best described by an algor mortis model for a sea lion with a mass of 24 kg. The gray diamond indicates the regional estimate for sea-surface temperature (for sources, see Table 1). The arrow indicates that the tag sensed light or air and began to transmit only 16 d post mortem.

of 7.55°C but did not sense light or air, and they did not commence transmissions until 5 and 9 d post mortem, respectively (Table 1; Fig. 5). The regional SST estimate of 4.97°C was more than 2°C below recorded postmortem temperatures, and it was closely matched by uplink temperatures of 5.22°C from 1 tag (5 d post mortem). The second tag was beached by the time of the first successful uplink (9 d post mortem) and reported air temperatures near freezing.

The 2 tags from the remaining event (TJ63) recorded disparate details that corresponded with type-I and -IV scenarios (Fig. 5). One tag (type I) recorded precipitous temperature drops (peak ΔT: −0.94°C min^{-1}) to 5.20°C, close to the regional SST estimate of 5.07°C (Table 1). This tag began making transmissions at the first opportunity after sensing air and light, reporting uplink temperatures of 5.73°C. The second tag (type IV), however, recorded a precipitous drop (peak ΔT: −0.90°C min^{-1}) to 7.02°C. It started transmissions only 6 d later, after sensing light or air, and reported temperatures of 5.13°C, a level close to the regional SST estimate of 4.73°C (Table 1).

Pairs of LHX tags without disparate fates (as indicated by light or air data) reported consistent postmortem temperatures: the mean difference within pairs (n=11) was 0.15°C (SD 0.13), the maximum difference was 0.51°C. Temperatures uplinked by tags free-floating at the sea surface also were consistent with regional SST estimates, with a mean difference within pairs (n=16) of 0.32°C (SD 0.31). The mean difference between postmortem temperatures and SSTs for tags without delayed transmissions (n=23) was 0.24°C (SD 0.21). However, the average (absolute) difference between regional SST estimates and postmortem temperatures reported by 4 tags that showed both precipitous temperature drops and delayed transmissions was considerably higher at 3.29°C (range: 1.95–6.05°C; Fig. 5). Each of these 4 tags also recorded postmortem temperatures that were extremely constant, with maximum temperature fluctuations of 0.0°C, 0.2°C, 0.0°C, and 0.0°C over a period of

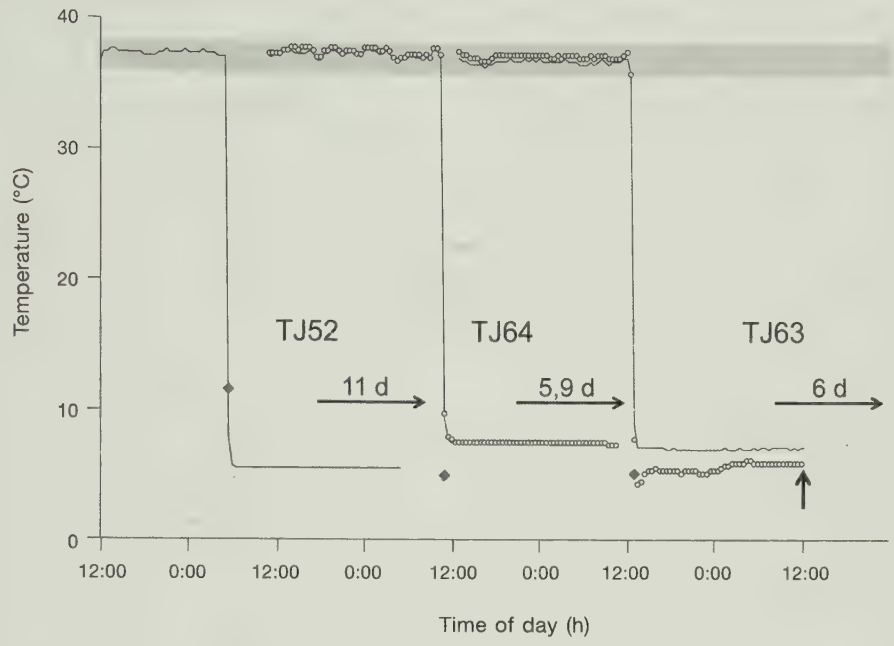

Figure 5

Postmortem temperatures indicating rapid cooling recorded for 3 mortalities of Steller sea lions (*Eumetopias jubatus*) and subsequently transmitted by life history transmitters (LHX tags) with delayed onset of transmissions. The 3 events occurred in the Gulf of Alaska in 2011. Temperatures were recorded by 5 tags in 30-min increments and subsequently transmitted through the Argos satellite system. Data were received from only 1 LHX tag for animal number TJ52. The left horizontal arrow indicates that this tag sensed light or air and began transmissions 11 days post mortem. Data were received from 2 LHX tags for animal TJ64 (line, circles). The middle horizontal arrow indicates that these tags sensed air or light and began to transmit 5 and 9 d post mortem. The right horizontal arrow indicates that one LHX tag from animal TJ63 (line) sensed light or air and began transmissions 6 d post mortem. The vertical arrow indicates when the second tag from the sea lion TJ63 (circles) began to transmit after sensing light or air. Gray diamonds indicate the regional estimates of sea-surface temperatures (for sources, see Table 1). The gray shaded area at the top of this graph indicates the normal range of abdominal temperatures for healthy sea lions (Fig. 1).

24 h post mortem. Conversely, 5 tags without delayed transmissions from hosts that died close to noon and, therefore, also provided 24 h of postmortem temperatures recorded maximum fluctuations of 0.82°C, 1.43°C, 1.83°C, 2.2°C, and 2.4°C (Table 1).

Discussion

We previously reported that survival rates based on data from LHX tags for juveniles of ages 14–60 months in our study were indistinguishable from control values from a separate study based on resightings of individuals that had been hot-iron branded (Horning and Mellish, 2012; see *Materials and methods* section). Our probability for event detection was very high at 98.4%, indicating that, most likely, no events went undetected. This high level of probability supports the hypothesis that LHX tags or surgeries do not affect postrelease survival to 60 months in implanted juvenile Steller sea lions. All 15 detected mortality events with data were classified as death by predation on the basis of precipitous temperature declines (14 events) or the very low mass indicated by the algor mortis model (1 event). In combination, these findings support the notion that none of the events reported here were caused by implanted tags or tagging procedures; after all, it would seem highly improbable that LHX tags could increase the susceptibility of sea lions to predation without a concurrent decrease in long-term survival rates below control values.

Theoretically, the observed patterns of rapid drops in temperature could be associated with direct extrusion of a tag by a live host animal. Noncatastrophic extrusion (i.e., percutaneous) is a slow process that should result in substantially different times for the

temperature drops recorded by the 2 implanted tags. No such differences were recorded in this study. Rapid, catastrophic extrusions (i.e., endogastric) are almost certainly lethal and would result in lower survival rates compared with the rates of control groups. We, therefore, deem direct extrusion as extremely unlikely.

It is also possible that rapid temperature drops resulted from tags liberated by scavengers that fed on a carcass within 30–60 min post mortem. Scavenging after a longer time would have revealed at least initial changes of cooling conforming to a type-II scenario (Fig. 2). Many processes that lead to death are associated with the reduced ability of marine mammals to thermoregulate (Dierauf and Gulland, 2001; Rosen et al., 2007; Stoskopf, 2005). Inflammation and infection often lead to pyrexia (fever). In extreme cases of septicemia, temperatures may even continue to rise post mortem. Hypoglycemia, starvation and emaciation, congestive cardiac failure, hemorrhage, and shock are all associated with a reduced body-core temperature. Furthermore, many injured or diseased pinnipeds have a tendency to come ashore and die there (Dierauf and Gulland, 2001), although this behavior may not apply to highly pelagic species, such as elephant seals. None of the observed events were associated with any signs of compromised health, such as pyrexia or hypothermia, and all events occurred at sea. Death from drowning (following entanglement) should lead to a type-III scenario that involves slightly accelerated, internal cooling by inhaled cold water, although not to the level exhibited in Figure 3 (in marine mammals, total lung capacity [in liters] is typically <10% of body mass [in kilograms]; Kooyman [1989]).

This evaluation of various possible cooling rates and antemortem temperatures indicates that, at onset of the rapid temperature drops shown in Figure 4 and 5, the animals were alive and healthy and that death likely coincided with rather than preceded releases of LHX tags. Therefore, although scavenging shortly after death could not be categorically excluded at this point, we found no evidence of nontraumatic death at sea and deem such an event unlikely. Predation is the most likely and consistent interpretation for all 15 events, which fit type-I, -III, or -IV scenarios, discussed here.

Tags from 11 type-I events quickly sensed light or air and did not have delayed transmissions. These tags reported postmortem temperatures that corresponded with regional SST estimates. These events may have happened at or near the sea surface, where tags were exposed quickly to ambient light while their antennas floated above water. Free-floating LHX tags ascended at a mean rate of 1.3 m s^{-1} (standard error of mean [SE] 0.05) during runs (n=10) simulated in laboratory trials (senior author, unpubl. data). Even tags liberated at a depth of 500 m, beyond the 300 m reported as the deepest dive depth for juvenile Steller sea lions (Mellish et al., 2007), still would reach the surface within 10 min, and, once there, they would record temperatures corresponding to the SST and the air surrounding their floating antennas, and ambient light levels would trigger transmissions. Therefore, no inferences can be made from these 11 type-I events on the species of the predator.

One type-III event yielded a gradual temperature decline from 1 tag with delayed sensing of light and air. The tag cooled to a temperature of 4.51°C, a level above the regional SST estimate of 3.7°C but similar to values for temperatures at a depth of approximately 50 m. This temperature pattern could be the result of the sinking of the LHX tag after dismemberment of the animal, with a portion of sea lion tissue surrounding the tag, to a depth where the slightly warmer water temperature was recorded. However, LHX tags are coated with a material designed to prevent connective tissue growth and encapsulation or adhesion to the omentum in the abdominal gut cavity (Horning and Hill, 2005). Temperature drops from all other tags indicated immediate and complete separation from all tissue, and all 9 tags that were physically recovered—including the tag from this type-III event—had no signs of connective tissue or adhesion. It is difficult to imagine that a 24-kg portion of abdominal tissue could remain after dismemberment and could contain a tag for an extended period of time without the tag being released. It seems more plausible that this LHX tag and some sea lion tissue were ingested together by a cold-bodied predator, resulting in gradual cooling in darkness.

In the 3 remaining events, 4 tags with precipitous drops and delayed onset of transmissions recorded temperatures different from SSTs but similar to estimates for temperatures at depths between 50 m (TJ63, TJ64) and 200 m (TJ52) for 24 h post mortem (Table 1; Fig. 5). These tags detected light or air only 5–11 d post mortem, at which time uplink temperatures corresponded with regional SST estimates. The combination of the absence of light or air and of extended recordings of deepwater temperatures indicates that these tags remained in the dark while surrounded by a cold, wet medium after their abrupt removal from the bodies of their hosts. In 1 event, only 1 of 2 tags recorded deepwater temperatures while remaining in the dark, but the other tag recorded postmortem temperatures that matched regional SST estimates and uplinked data immediately after sensing light and air (Fig. 2). The postmortem temperatures recorded by each of the 4 tags with delayed uplinks essentially were constant, in comparison with data from tags that floated at the surface and recorded temperature fluctuations between 0.82°C and 2.40°C over a 24-h period.

The most parsimonious explanation for our data is that the 4 tags with precipitous temperature drops and delayed onset of transmissions were ingested after an attack by a cold-bodied predator. Tag ingestion simultaneously explains absence of air and light, delayed onset of transmissions, and constant postmortem temperatures distinct from SSTs. Three poikilotherm species have been reported as potential predators of Steller sea lions (Loughlin and York, 2000): the white shark, the

salmon shark, and the Pacific sleeper shark. All 3 species have been documented feeding within Alaskan waters and the Bering Sea (Compagno, 1984; Benz et al., 2004; Martin, 2005). However, lamnid sharks, including the salmon shark (Smith and Rhodes, 1983; Anderson and Goldman, 2001; Goldman et al., 2004; Bernal et al., 2005) and the white shark (McCosker, 1987; Goldman, 1997), have been shown to maintain body-core and stomach temperatures consistently 6–15°C above ambient temperatures even in subarctic regions with SSTs below 5°C. Therefore, the Pacific sleeper shark emerges as the only potential predator of Steller sea lions in the North Pacific and Bering Sea region whose body-core temperatures likely remain at mid-water values.

The postmortem ingestion by Pacific sleeper sharks of tags released through the attack of a different predator is another possible interpretation. However, a delayed secondary ingestion at the surface still would expose LHX tags to SSTs and ambient light, and the probability of a simultaneous encounter of a sea lion and 2 distinct predator species at depth would be very low, in part because it would expose Pacific sleeper sharks to predation risk (Ford et al., 2011).

The Pacific sleeper shark is found in the North Pacific and the Bering Sea. Pacific sleeper sharks have been documented in shallow coastal waters and even in estuarine waters in the arctic regions of the North Pacific and at oceanic depths (>2700 m) in temperate latitudes (Compagno, 1984; Ebert et al., 1987; Hulbert et al., 2006; Sigler et al., 2006; Yano et al., 2007). Although the very large (up to 7 m in total length [TL]) Greenland shark (*Somniosus microcephalus*) may have the northernmost range documented for species of this genus, the range of the Pacific sleeper shark extends into the Bering Sea and past the Arctic Circle (Bright, 1959; Orlov, 1999; Orlov and Moiseev, 1998, 1999; Benz et al., 2004; Hulbert et al., 2006; Courtney and Sigler, 2007).

Pacific sleeper sharks have been documented to reach adult lengths up to 4.4 m TL (Compagno, 1984; Mecklenburg et al., 2002; Yano et al., 2007), but estimates from historic and contemporary photographic and video recordings indicate that they may reach lengths in excess of 7 m TL (Hart, 1973; Compagno, 1984). The estimated TL at sexual maturity is 3.7 m for females and 4.0 m for males (on the basis of the length of the few sexually mature individuals ever caught: Ebert et al., 1987; Courtney and Sigler, 2007; Yano et al., 2007). To date, only 5 mature females have been reported from bycatch[2]. Pacific sleeper sharks are slow growing and long-lived (Ebert et al., 1987), supporting the notion that mature adults should reach sizes well beyond 4–5 m TL.

[2] Tribuzio, C. A., K. Echave, C. Rodgveller, J. Heifetz, and K. J. Goldman. 2010. Assessment of the sharks in the Bering Sea and Aleutian Islands. In stock assessment and fishery evaluation report for the groundfish resources of the Bering Sea/Aleutian Islands regions, p. 1451–1500. [Available from North Pacific Fishery Management Council, 605 West 4th Ave., Suite 306, Anchorage, AK 99501.]

Pacific sleeper sharks have a broad range of diet that includes benthic and mid-water crustaceans, cephalopods, salmonids, gadids, flatfish, and marine mammals (Bright, 1959; Compagno, 1984; Yang and Page, 1998; Orlov, 1999; Orlov and Moiseev, 1998, 1999; Sigler et al., 2006; Yano et al., 2007). Genetically identified stomach contents have included cetaceans and phocids; however, a histological analysis of ingested tissue indicated scavenging rather than live captures in the case of all cetacean and some phocid samples, and the evidence was inconclusive for some freshly ingested phocid tissue (Sigler et al., 2006).

Pacific sleeper sharks may appear to be improbable predators of fast-swimming and agile prey as large as juvenile Steller sea lions, especially given the adaptation of the functional morphology of their feeding apparatus toward suction feeding. Evidence shows that Pacific sleeper sharks have removed large circular sections, cookie-cutter style, from whale carcasses (Barrett-Lennard et al., 2011). Such an injury, if inflicted on the lower abdominal area (between diaphragm and pelvic girdle) of large juvenile sea lions, could result in LHX tag ingestion and would be lethal if it occurred ante mortem.

Several studies of shark predation on marine mammals (Arnold, 1972; Cockroft et al., 1989; Heithaus, 2001; Bertilsson-Friedman, 2006) have indicated that predation attempts often involve approaches from below and the rear and may focus on abdominal areas. However, the attack behavior of the Pacific sleeper shark has not been studied. Pacific sleeper sharks do appear capable of preying on fast-swimming salmonids and scombrids (Ebert et al., 1987; Sigler et al., 2006). Larger and older Greenland sharks have been shown to prey on live pinnipeds (Fisk et al., 2002; Leclerc et al., 2012), although such events may be ambush attacks on seals sleeping at the ocean surface (Watanabe et al., 2012). Southern sleeper sharks (*Somniosus antarcticus*) have been reported to attack live southern elephant seals (*Mirounga leonina*) (Van Den Hoff and Morrice, 2008).

Because of the potential habitat overlap, the role of the Pacific sleeper shark as a potential predator of Steller sea lions was investigated. A modeling exercise by Frid et al. (2009) revealed sea lion dive behavior as avoidance of predation risk from transient killer whales and Pacific sleeper sharks. The model output was supported by telemetered data of sea lion dive behavior in Prince William Sound. Yet, no sea lion remains were identified in the stomachs of 198 Pacific sleeper sharks caught by long-lining near sea lion rookeries (Sigler et al., 2006). However, these 198 sharks were collected during 2 sampling periods in May and August and within 1–12 km of sea lion rookeries or haulouts (Sigler et al., 2006).

Only 3 of the 15 predation events that were detected in our study occurred within the 4-month period of May–August (Table 1), and none of these 3 events occurred within 20 km of rookeries or haulouts. Fur-

thermore, the absence of sea lion remains could be explained by the comparably small size (1.3–2.8 m TL) of the sharks sampled, if smaller sharks are less likely to attack larger and faster prey items. All sampled animals were probably immature (Courtney and Sigler, 2007). This size bias could be driven by the possible absence of larger sharks in the region (Hulbert et al., 2006) or by the possible size bias of long-lining toward younger, smaller individuals (for remarks on landing and sampling likelihood for long-line gear, see Orlov and Moiseev, 1999; Yano et al., 2007; Courtney and Sigler, 2007). Courtney and Foy (2012) reported an increase in trophic position with TL in Pacific sleeper sharks. They suggested that the diet of the Pacific sleeper shark probably also varies by time and location of capture and in response to prey availability. Therefore, given the evidence of ontogenetic, seasonal, and geographic diet shifts in many shark species, including the Greenland shark, southern sleeper shark, and Pacific sleeper shark (Orlov and Moiseev, 1999; Fisk et al., 2002; Yano et al., 2007; Courtney and Foy, 2012), the absence of sea lion tissue in the sampled sharks is, perhaps, not surprising and does not contradict our indirect evidence of predation on Steller sea lions.

Conclusions

Of all observed events of predation on juvenile Steller sea lions, at least 3 and likely 4 in 15—or approximately 27%—could be attributed to Pacific sleeper sharks. Although these observations do not constitute proof of attacks on live Steller sea lions by Pacific sleeper sharks, our data indicate that Pacific sleeper sharks need to be considered as a possible source of mortality of juvenile Steller sea lions in the region of the Gulf of Alaska and Prince William Sound.

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Age and growth of dusky grouper (*Epinephelus marginatus*) (Perciformes: Epinephelidae) in the southwestern Atlantic, with a size comparison of offshore and littoral habitats

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Abstract—We examined the age and growth of dusky grouper (*Epinephelus marginatus*) at offshore Carpinteiro Bank (32°16′S; 52°47′W) in the southwestern Atlantic through the analysis of growth increments in otoliths. We also examined the hypothesis that this offshore habitat represents a superior site for fish growth compared with inshore habitats. Samples consisted of 211 groupers captured by small-scale fisheries between 2008 and 2011 and with total lengths ranging from 150 to 1160 mm. Otolith growth increments were deposited once per year, and opaque bands formed mostly during the summer, as determined through marginal increment analysis. Ages ranged from 1 to 40 years, and most fish were aged to be between 2 and 8 years (global mean: 7.4 years, standard deviation 6.9). Von Bertalanffy growth parameters for pooled sexes were L_∞=900.9 mm, K=0.129 and t_0=−1.45. Fish from the offshore habitat were generally older and their mean sizes were significantly larger at ages 3, 4, 5, and 6 than those of fish from the inshore habitat. Our findings highlight the importance of Carpinteiro Bank as an important growth habitat for dusky groupers in the southwestern Atlantic and indicate that fishery management measures should be implemented to ensure the maintenance of this endangered species in this offshore bank in the future.

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The dusky grouper (*Epinephelus marginatus*) (Epinephelidae) inhabits shelter-rich, hard substrata on the continental shelf along the eastern coast of South America from Brazil to Argentina (Figueiredo and Menezes, 1980; Irigoyen et al., 2005), throughout the Mediterranean Sea, and from the British Isles around the southern tip of Africa (Heemstra and Randall, 1993) to southern Mozambique (Fennessy, 2006). Adults inhabit rocky bottoms, generally at depths up to 50 m, and they can be found occasionally at depths up to 250 m (Bruslé, 1985; Heemstra and Randall, 1993), whereas juveniles are usually found in coastal tide pools (Azevedo et al., 1995). Like other epinephelids, the dusky grouper is a slow-growing protogynous hermaphrodite species with late sexual maturation and low natural mortality rates (Manooch and Mason, 1987), and it is also a highly valuable commercial fish (Heemstra and Randall, 1993). These combined characteristics make the dusky grouper highly vulnerable to overfishing. This species is classified as endangered by the International Union for Conservation of Nature (Cornish and Harmelin-Vivien, 2004), with a decreasing global population trend.

The dusky grouper is the only large epinephelid that is usually found along the extreme southern part of the Brazilian coast, where it is fished over inshore rocky bottoms (depths <5 m) and offshore banks (depths >10 m) by small-scale fisheries (Condini et al., 2007). Offshore populations of dusky grouper occasionally are targeted opportunistically by large-scale commercial fisheries that use hand lines, but the species is otherwise not a commercially targeted species. The most frequently visited site in the southwestern Atlantic for commercial offshore fishing of this species is Carpinteiro Bank.

The role of dusky groupers in the coastal ecosystems of South America is not completely understood. Given the likely importance of this species as a predator (Reñones et al., 2002), it is imperative to understand the implications of any sort of commercial exploitation that may be involved. To prevent stock collapse, reef fishes should be fished carefully because slow-growing, late-maturing fish species are found frequently in coastal ecosystems and are vulner-

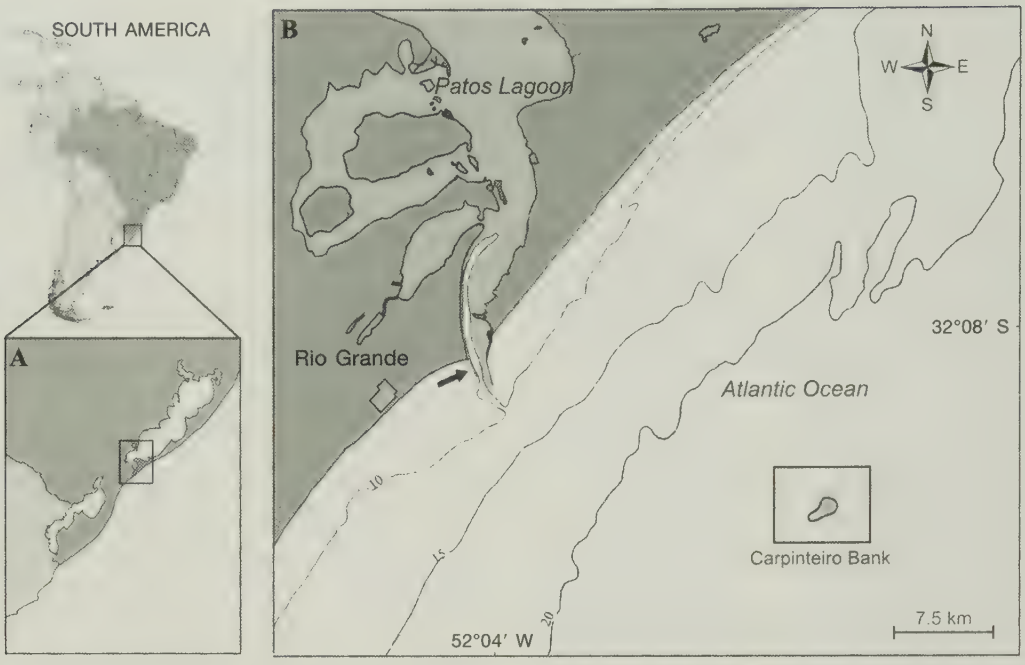

Figure 1

Map showing (A) Patos Lagoon and its estuarine zone (10.36 km^2) in Rio Grande, the southernmost state in Brazil; (B) the location of the pair of rocky jetties at the mouth of the lagoon (arrow) and, in the adjacent marine area, Carpinteiro Bank in the southwestern Atlantic. The lines along the coast denote the 10-, 15- and 20-m isobaths. The specimens of the dusky grouper (Epinephelus marginatus) sampled in this study were caught at the Carpinteiro Bank by small-scale fisheries from 2008 to 2011.

able particularly to overfishing (Coleman et al., 2000; Sadovy et al., 2013). Quantifying the age and growth of dusky groupers is required, therefore, to support adequate conservation and management of this species at its southernmost region of occurrence.

Previous investigations have provided initial information on the age structure of the dusky grouper in South America and also revealed the absence of reproduction in a coastal population associated with littoral rocky jetties from extreme southern Brazil (Seyboth et al., 2011). Those authors adopted the classification developed by Craig and Hastings (2007) that placed the dusky grouper in *Mycteroperca*. For our study, we followed the most recent classification (Craig et al., 2011), in which the species was retained in *Epinephelus*, given the absence of conclusive studies to change the genus. Another study conducted in this region revealed that the dusky grouper reproduces at offshore Carpinteiro Bank (Condini et al., 2014).

In this study, we validated the timing of increment deposition in otoliths and examined the aging structure and growth parameters of dusky grouper in an offshore habitat (Carpinteiro Bank, at depths of 15–25 m). Because we had access to the original data of Seyboth et al. (2011), we also aimed to test the hypothesis, previously reported by Seyboth et al. (2011), that offshore habitats (depths of 15–25 m) may constitute better habitats for growth and reproduction of dusky groupers than inshore habitats (depths <5 m).

Material and methods

Sampling, fish processing, and study area

Dusky grouper samples examined in this study (n=211) were identified according to Heemstra and Randall (1993). They were collected twice a month from landings in small-scale fisheries in Rio Grande, a port city in southern Brazil (Fig. 1), from 2008 to 2011. Fish were caught with hand lines, and their origin was evaluated through interviews with owners of fishing boats. Only fish caught specifically at the Carpinteiro Bank were considered. In the laboratory, the total length (TL) in millimeters and weight (WT) in grams of each specimen were measured, and sex was identified by a macroscopic examination of the gonads following Marino et al. (2001). Male and female gonadal differences are quite clear in macroscopic examination for this hermaphroditic grouper (Marino et al., 2001; Condini et al., 2014). The Carpinteiro Bank (32°16 S; 51°47 W) (Fig. 1) is a submersed reef, with depths ranging from 15 to 25 m, formed by beach rocks and is superficially

colonized by bryozoans, sponges, crustaceans, polychaetes, and coralline algae (Buchmann et al., 2001). Located approximately 30 km offshore from the city of Rio Grande (32° S), this bank is likely the most austral region where the dusky grouper is commercially exploited.

Otolith processing

The sagittal otoliths were dissected from all samples and stored dry. The otoliths were then embedded in epoxy resin and sectioned through the core with an Isomet[1] low speed saw (Buehler, Lake Bluff, IL) coupled to a diamond wafering blade to obtain sections 0.4–0.6 mm thick. These sections were glued to histological glass slides, polished with fine sandpaper (grits of 2000–8000), embedded in Entellan mounting medium (Merck KGaA, Darmstadt, Germany), covered with thin glass slides, and, finally, examined under a stereomicroscope coupled to a computerized imaging system. All sections were photographed under transmitted light so that translucent and opaque bands in the otoliths could be appear as white and dark bands in the digital photographs, respectively. Each otolith was read 3 times at intervals of one month by an experienced reader with no prior knowledge of either fish size or previous reading results. Only those otoliths with at least 2 corresponding increment numbers were used in our study.

The precision of otolith readings was evaluated through analysis of average percent error (APE; Campana, 2001) with the following equation:

$$APE = 100\% \times \frac{1}{R} \sum_{i=1}^{R} \frac{\left| x_{ij} - x_j \right|}{x_j}, \quad (1)$$

where x_{ij} = the ith age determination of the jth fish;
x_j = the mean age estimate of the jth fish; and
R = the number of times that each fish is aged.

Validation and growth

The timing of otolith incremental deposition was estimated through marginal increment analysis (MIA) following Manickchand-Heileman and Phillip (2000), with the extra growth expressed as a proportion of the previous year's growth:

$$MIA = (R_t - R_t{-}1) / (R_t{-}1 - R_t{-}2), \quad (2)$$

where R_t = the distance measured from the core to the otolith's edge;
$R_t{-}1$ = the distance between the core and the distal margin of the last opaque band; and
$R_t{-}2$ = the distance between the core and the distal margin of the penultimate opaque band.

[1] Mention of trade names or commercial companies is for identification purposes only and does not imply endorsement by the National Marine Fisheries Service, NOAA.

Growth increment measurements were conducted with otolith images by using the free software ImageTool (University of Texas Health Science Center, San Antonio), which was calibrated with a micrometric scale glass. For MIA, 188 otoliths (from fish of sizes from 277 to 1160 mm TL) were used. During summer, autumn, winter, and spring, 91, 48, 10, and 39 otoliths, respectively, were obtained,. Marginal increment values were averaged over the summer (January–March), autumn (April–June), winter (July–September) and spring (October–December). These averages were compared by using the nonparametric Kruskal-Wallis and Mann-Whitney tests (Zar, 1999) because the data were not normally distributed.

To estimate average growth, the number of otolith increments was modeled against TL by fitting a von Bertalanffy (Eq. 3) curve and by using a nonlinear least squares method (Ricker, 1975).

$$TL = L_\infty (1 - e^{-K(t-t_0)}), \quad (3)$$

where TL = the TL corresponding to age I;
L_∞ = the mean theoretical maximum TL;
t_0 = the theoretical age at zero length; and
K = the growth coefficient, the rate at which the curve approaches the asymptote.

These parameters were estimated both on the basis of all samples (male+female) and on the basis of females only. Growth parameters were not estimated for males because of their small sample size (n=11).

Sea-surface temperatures

Data on sea-surface temperatures (SSTs) were analyzed from a 40-year time series measured in the region neighboring the Carpinteiro Bank (specifically, between 32–32°50′S and 50–52°W) made available by the Coastal and Estuarine Oceanography Laboratory of Rio Grande Federal University. SST data were averaged by season. Data on subsurface temperature were not available for this site.

Framework for habitat comparison

The hypothesis that offshore habitats may provide better environmental conditions for growth of dusky groupers than inshore habitats was tested by comparing mean sizes between fish from both sites. Specifically, the mean sizes at ages 3, 4, 5, 6, and 7 of fish collected offshore at the Carpinteiro Bank (depths of 15–25 m) were compared with the mean sizes of fish collected from the inshore rocky jetties of Rio Grande (depths <5 m) from 2007 to 2009 that were previously reported by Seyboth et al. (2011). These age groups were selected on the basis of the simultaneous availability of 10 or more individuals per age group at both sites. Length-at-age data of inshore dusky groupers (Seyboth et al., 2011) were made available by those authors. Otoliths in this study and in Seyboth et al. (2011) were read

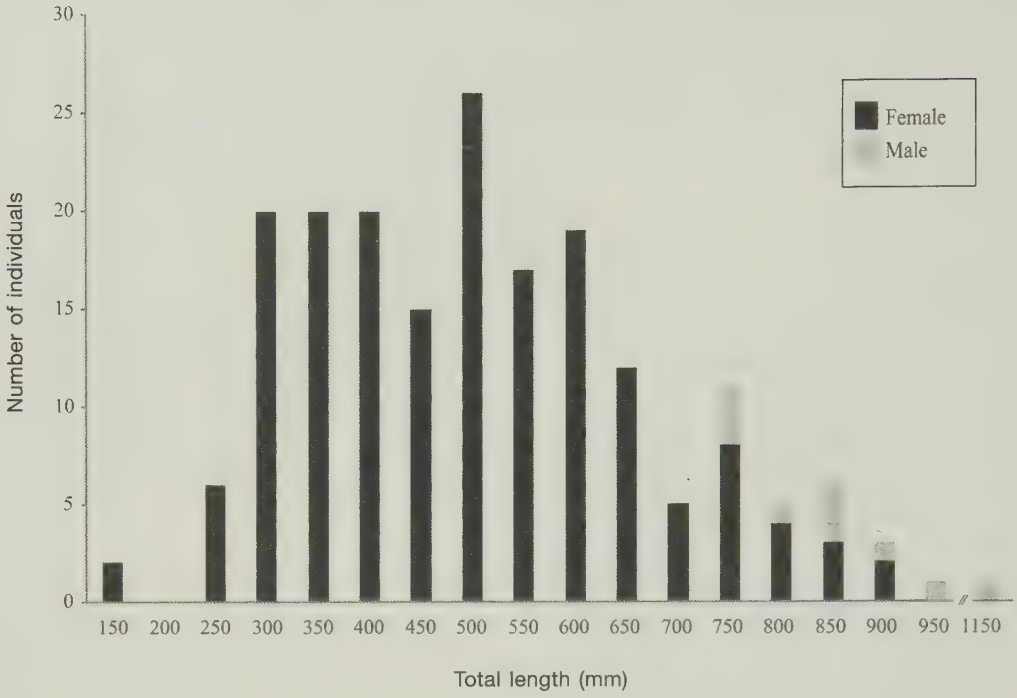

Figure 2

The total lengths, measured in millimeters, of female (black bars) and male (gray bars) dusky groupers (*Epinephelus marginatus*) caught at Carpinteiro Bank in the southwestern Atlantic (n=189) by small-scale fisheries from 2008 to 2011.

by the same person. Statistical analysis was conducted through a one-way analysis of variance after verification of the assumptions of normality and homoscedasticity (Zar, 1999).

Results

The TL of the dusky groupers sampled at Carpinteiro Bank ranged from 150 to 1160 mm TL (Fig. 2), and 80% of all samples measured between 300 and 700 mm TL. Females (n=200) accounted for the vast majority of samples, whereas males (n=11) composed only 5.5% of all samples. As expected, the mean TL for males (890.2 mm TL [standard deviation (SD) 122]) was larger than the mean for females (512.1 mm TL [SD 152.9]). The length–weight relationship was $WT = 0.00001 \times TL^{3.094}$ (coefficient of determination [r^2]=0.985), and the allometric coefficient was significantly different from 3 (t-test: P<0.0003), revealing that the species grows a little more in weight than in length.

The opaque and translucent growth bands in the otoliths of the dusky grouper were reasonably discernible, resulting in fine levels of precision during the process of otolith reading (APE=6.9%, n=211). Of the 211 examined otoliths, 22 were excluded from the analysis because of the absence of agreement among readings and 188 and 189 were used to validate the timing of deposition of growth increments and to estimate aging structure and growth, respectively.

The mean distribution of marginal increments in otoliths had significant differences among the fish collected during the 4 seasons (Kruskal-Wallis test: P<0.03; Fig. 3A). Marginal increment values were significantly lower during the winter than during the spring and summer (Mann-Whitney test: P<0.005 and P<0.018, respectively), and this pattern of differences was the same as the one observed for the proportion of opaque bands in the otolith edges (Fig. 3B). The same pattern was obtained for mean SST, where the proportion of opaque bands at the otolith edges was positively correlated with SST (r^2=0.841).

Ages ranged from 1 to 40 years, and 85% of samples were aged between 2 and 8 years (Table 1). The mean ages for males and females were 26.7 years (SD 6.3) and 6.1 years (SD 4.9), respectively. The youngest sampled male was 20 years old and measured 760 mm TL, whereas no females were found at ages of more than 27 years or lengths greater than 933 mm TL. The von Bertalanffy parameters that were estimated for pooled sexes (n=189; L_∞=900.9 mm TL, K=0.129, t_0=−1.45 years) showed smaller values for L_∞ and larger values

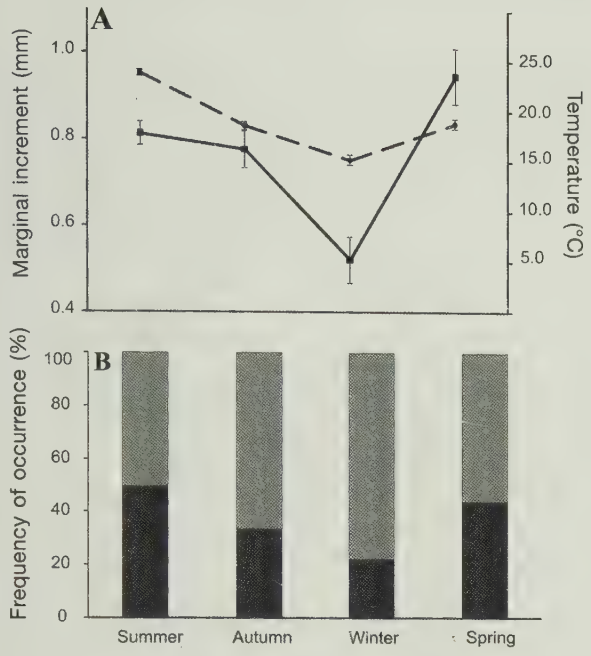

Figure 3

Seasonal variation (**A**) in the mean marginal increment (solid lines) and sea-surface temperature (dashed line) and (**B**) in the frequency of occurrence of opaque (black bars) and translucent edges (gray bars) of otoliths of dusky groupers (*Epinephelus marginatus*) caught at Carpinteiro Bank in the southwestern Atlantic by small-scale fisheries from 2008 to 2011 (summer: n=91; autumn: 48; winter: 10; and spring: 39). Error bars in panel A indicate ±1 standard error of the mean.

for K when males (n=11) were removed from the analysis (n=178, L_∞=851.1 mm TL, K=0.153, t_0=−1.06 years). The von Bertalanffy curve had the typical exponential asymptotic shape and allowed us to estimate that 70% of the asymptotic length of a fish was achieved at approximately 8 years of age (Fig. 4; Table 1).

A comparison of fish sizes at ages 3–6 between littoral sites and offshore sites showed that dusky groupers from the offshore Carpinteiro Bank were significantly larger than fish from the inshore rocky jetties located at the mouth of Patos Lagoon in the Rio Grande (F=22.39, P<0.001; Fig. 5), with mean differences of 11.7%, 19.6%, 16.7%, and 11% per year, respectively. Significant differences, however, were not observed for age 7.

Discussion

For otoliths to be used as reliable age indicators, they must display an internal structure of increments formed on a regular and predictable time scale. In dusky groupers, otoliths present well-defined growth increments that allow for age estimation at a relatively high precision. Translucent and opaque bands typically were formed during winter and summer, respectively, as determined through the analysis of marginal increments. Marginal increment analysis, however, is not considered the most trustworthy technique for validation of growth increments in otoliths (Campana, 2001), particularly where the temporal variation in environmental temperature is not strong enough to significantly influence fish metabolism. Therefore, to help increase confidence in validation, the seasonality of SST also was examined (Beckman and Wilson, 1995; Fablet et al., 2011). As expected, the results from marginal increment analysis closely followed the seasonal fluctuations in SST—a finding that supports the hypothesis that opaque and translucent increments in the otoliths of dusky groupers may be formed on an annual basis.

Moreover, the annual deposition of growth increments in otoliths has been reported previously for the dusky grouper (Fennessy, 2006; Reñones et al., 2007) and other grouper species, such as the yellowedge grouper (*E. flavolimbatus* [Manickchand-Heileman and Phillip, 2000]), yellowmouth grouper (*Mycteroperca interstitialis* [Manickchand-Heileman and Phillip, 2000]), leopard grouper (*M. rosacea* [Díaz-Uribe et al., 2001]), orange-spotted grouper (*E. coioides* [(Grandcourt et al., 2005)], and island grouper (*M. fusca* [Bustos et al., 2009]), indicating that it is an usual characteristic in epinephelids. The same pattern also was found for other long-lived fish species, such as the whitemouth croaker (*Micropogonias furnieri* [Haimovici and Umpierre, 1996]) and wreckfish (*Polyprion americanus* [Peres and Haimovici, 2004]), that inhabit the southwestern Atlantic.

The first finding on the growth of the dusky grouper in this study was the absence of males younger than 20 years or smaller than 760 mm TL—probably a result of protogynous hermaphroditism in this species (Heemstra and Randall, 1993; Andrade et al., 2003; Reñones et al., 2010; Condini et al., 2013). Under that reproductive strategy, dusky groupers are expected to first mature as females (at approximately 5 years old) and then change sex into males after approximately 7 years old (Reñones et al., 2010). Results from the few studies that have examined age and gonads simultaneously in dusky groupers indicate that males first appear in dusky grouper populations at diverse sizes and ages. For example, younger sampled males varied from 7 years (and ~580 mm TL) in the Mediterranean Sea (Reñones et al., 2007) up to 9 years (and ~810 mm TL) off southeast Africa (Fennessy, 2006). We recognize that variables like sampling selectivity, environmental conditions, intraspecific competition, and fishing pressure may be related to the age and size at which sexual transition occurs. However, at this point, we have no further evidence to explain the very low abundance of male dusky groupers found in our study.

Table 1

Ages, number of specimens sampled (n), and means and ranges of total length (TL) and weight (WT) for dusky groupers (*Epinephelus marginatus*) caught at the Carpinteiro Bank in the southwestern Atlantic by small-scale fisheries from 2008 to 2011. Standard deviations of the means (SD) are provided in parentheses.

Age (years)	n	Mean TL (mm)	TL range (mm)	Mean WT (g)	WT range (g)
1	2	160.0 (14.1)	150–170	58.2 (14.6)	47.9–68.5
2	27	325.7 (38.0)	290–405	602.3 (196.6)	314.5–1227.9
3	28	385.9 (38.0)	277–453	1081.2 (297.6)	400.0–1675.8
4	28	464.1 (48.4)	365–561	1890.8 (661.5)	1015.0–3513.0
5	15	526.9 (44.4)	420–635	2806.0 (977.9)	1284.0–5902.0
6	22	563.9 (45.2)	485–680	3415.8 (1053.9)	2108.7–6530.0
7	21	587.3 (60.6)	475–671	4135.1 (1384.0)	2103.3–7045.0
8	12	658.1 (38.6)	606–713	5824.9 (1553.5)	4080.0–8950.0
9	1	532.0	532.0	3171.0	3171.0
10	3	709.7 (57.6)	650–765	7213.3 (2725.1)	4590.0–10030.0
11	2	744.0 (50.9)	708–780	7137.5 (88.4)	7075.0–7200.0
14	3	748.3 (72.9)	665–800	7785.8 (2671.2)	4725.8–9651.5
15	2	774.0 (28.3)	754–794	9390.0 (3620.4)	6830.0–11,950.0
17	2	729.5 (36.1)	704–755	6715.0 (792.0)	6155.0–7275.0
18	3	762.3 (87.9)	668–842	7746.7 (3225.9)	5100.0–11,340.0
19	1	764.0	764.0	7025.0	7025.0
20	1	850.0	850.0	10,000.0	10,000.0
21	1	770.0	770.0	8100.0	8100.0
22	2	825.0 (7.1)	820–830	10,900.3 (423.8)	10,600.6–11,200.0
23	5	808.0 (118.3)	660–985	10309.0 (4650.2)	6030.0–17,850.0
24	2	881.0 (12.7)	872- 890	10657.5 (2605.7)	8815.0–12,500.0
25	1	933.0	933.0	15,100,0	15,100.0
27	1	910.0	910.0	16,300.0	16,300.0
28	1	864.0	864.0	11,100.0	11,100.0
29	1	917.0	917.0	14,335.0	14,335.0
31	1	875.0	875.0	10,560.0	10,560.0
34	1	981.0	981.0	11,700.0	11,700.0
40	1	1160.0	1160.0	25,000.0	25,000.0

Examination of the von Bertalanffy parameters revealed that dusky groupers from Carpinteiro Bank grow slightly faster and attain slightly smaller theoretical maximum lengths than fish sampled from other areas in other studies. Although the von Bertalanffy model provided realistic estimates of growth parameters, L_∞ (900 mm TL) was considerably smaller than the maximum length actually observed in this study (1160 mm TL) because fish older than 25 years were poorly represented. Given that the growth parameters were estimated by least squares fitting, the addition of more old individuals would most likely increase L_∞ and decrease K because of the intrinsic antagonism of these parameters, and the revised set of parameters would be closer to the values reported in Table 2. Therefore, our results indicate that fitting a von Bertalanffy model to our actual data tends to slightly overestimate K and underestimate L_∞ compared with a more realistic sampling size. Also, it may be possible that K estimated for the Mediterranean Sea (see Table 2) were higher than the values for other areas because of limited sampling.

Groupers are generally recognized as long-lived fishes capable of reaching maximum ages of more than 60 years (e.g., Reñones et al., 2007). The maximum longevity of dusky groupers in this study was approximately 40 years, which is the second-highest longevity among the values observed worldwide (see Table 2). Currently, the most important factor influencing fish longevity most likely is fishing activity because fishing is expected to preferentially remove large, old fish from natural populations (King, 1995). According to local traditional fishermen, fisheries over the Carpinteiro Bank are known to have occurred since the 1960s, and fishing effort has been rising ever since. Additionally, fishing effort and the number of fishing boats operating in the region have increased during the last 3 decades (Cowx et al., 1998), and those changes could have resulted in the decreased availability of the older age classes of dusky grouper that we observed in this study. Another interesting finding was that approximately 40% of the fish captured offshore were immature (L_{50}=496 mm TL, as estimated from Condini et al., 2014). Given the com-

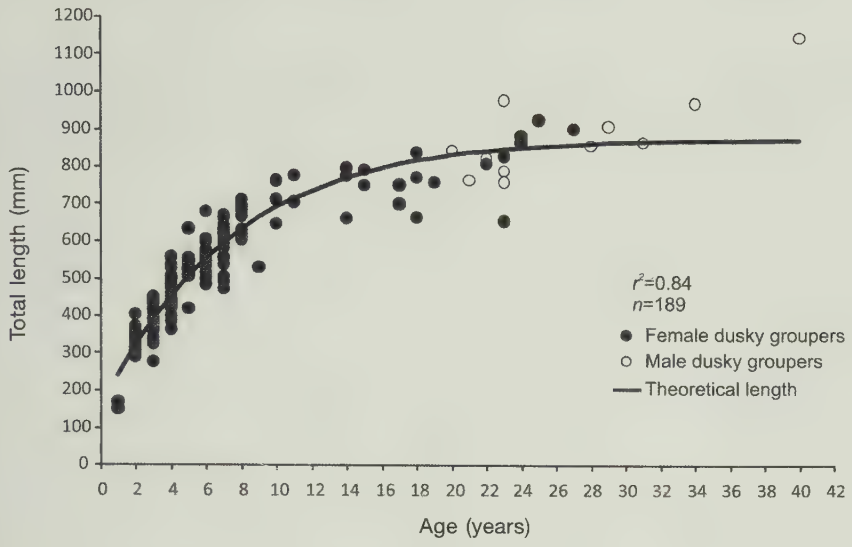

Figure 4

Total lengths, measured in millimeters, at ages and a fitted von Bertalanffy growth curve for dusky grouper (*Epinephelus marginatus*) specimens (n=189) caught at the Carpinteiro Bank in the southwestern Atlantic by small-scale fisheries from 2008 to 2011. r^2=coefficient of determination.

bined evidence of the low availability of old fish with the rising fishing effort over the last 3 decades and the large proportion of young adult individuals sampled here, it is reasonable to suggest that this offshore population may be undergoing overfishing. Unfortunately, there are no official fish landing records to allow an estimation of capture trends over time. These data would be necessary to objectively infer the population status of dusky grouper in southern Brazil.

The sampling conducted in the our study was restricted to Carpinteiro Bank, and that limit may cause concern because it is shallower (15–25 m) than the depths (up to 40–50 m) where larger groupers usually live (Heemstra and Randall, 1993). It is, therefore, plausible to argue that ontogenetic migration, which is usual in epinephelids (Dahlgren and Eggleston, 2000), could be withdrawing large-size (>90 cm) fish from the examined population because of their emigration toward waters with depths of 40–50 m. However, we posit that this notion is not the case for two main reasons. First, the continental shelf at the extreme southern part of Brazil basically is formed by sand bottoms, where reefs and rocks are scarce (Calliari and Klein 1993). There are just a few rocky banks that may provide habitat for groupers, and they are found specifically between the 20- to 30-m isobaths (Cardoso and Haimovici, 2011). Among these banks, Carpinteiro, Solidão, and Torres are the most visited by fishing boats (Cardoso and Haimovici, 2011). Second, reproduction has been observed for dusky grouper in Carpinteiro Bank (Condini et al., 2014), supporting the idea that the whole life cycle of the examined population occurs in this bank. Given these arguments, we believe that the relatively low abundance of older (>15 years) individuals is an in-

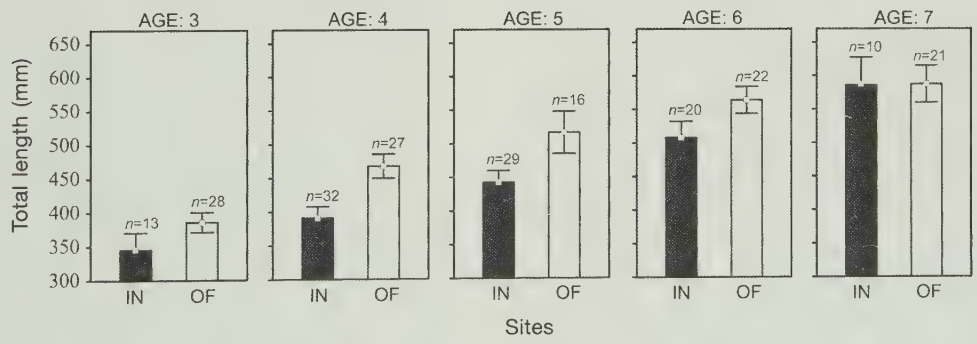

Figure 5

Comparison by age (3–7 years old) of average total length of dusky groupers (*Epinephelus marginatus*) collected from inshore (IN, black bars) and offshore (OF, open bars) habitats. Fish were collected from the inshore site, littoral rocky jetties near Patos Lagoon, from 2007 to 2009 and from an offshore site of Carpinteiro Bank from 2008 to 2011. Error bars indicate 95% confidence intervals.

Table 2

Study site, aging structure, range of total lengths (TLs), age range, number of specimens sampled (n), and von Bertalanffy growth parameters of dusky groupers (*Epinephelus marginatus*) examined in this study and in previous investigations. Ageing structures used were scales (Sc), sectioned otolith (So), and whole otolith (Wo). Von Bertalanffy growth parameters included L_∞, the mean theoretical maximum TL; t_0, the theoretical age at zero length; and K, the growth coefficient or the rate at which the curve approaches the asymptote.

Reference	Study site	Aging structure	TL range (mm)	Age range (years)	n	L_∞ (mm)	K (years^{-1})	T_0 (years)
Rafail et al. (1969)[1]	Mediterranean Sea —Egypt	Sc	166–475	1–7	251	800	0.112	−1.08
Chauvet (1988)[2]	Mediterranean Sea —Tunisia	Sc/Wo	53–1180	0–36	270	1144	0.090	−0.75
Kara and Derbal (1995)[3]	Mediterranean Sea —Algeria	Sc	197–567	1–7	41	785	0.160	−0.73
Bouchereau et al. (1999)[4]	Mediterranean Sea —France	Sc	200–1200	1–14	22	1359	0.080	−0.80
Fennessy (2006)[5]	Southern Western Indian —FranceSouth Africa	So	95–1020	1–16	215	1249	0.090	−1.43
Reñones et al. (2007)[6]	Western Mediterranean —Spain	So	66–1056	0–61	358	955	0.087	−1.12
Seyboth et al. (2011)[7]	Southern Western Atlantic —Brazil	So	260–800	2–12	108	1249	0.069	−1.49
Present study	Southern Western Atlantic —Brazil	So	150–1160	1–40	190	900	0.129	−1.45

[1] Rafail S. Z., W. L. Daoud, and M. M. Hilal. 1969. Long line Mediterranean fisheries studies west of Alexandria. GFCM Stud. Rev. 42, 16 p.

[2] Chauvet, C. 1988. Étude de la croissance du mérou *Epinephelus guaza* (Linné, 1758) des côtes tunisiennes. Aquat. Living Resour. 1:277–288.

[3] Kara M. H., and F. Derbal. 1995. Morphométrie, croissance et mortalité du Merou *Epinephelus marginatus* (Serranidae) des côtes de l'est algérien. Cah. Biol. Mar. 36:229–237.

[4] Bouchereau J. L., P. Body, and C. Chauvet. 1999. Growth of the dusky grouper *Epinephelus marginatus* (Linnaeus, 1758) (Teleostei, Serranidae), in the natural marine reserve of Lavezzi Islands, Corsica, France. Sci. Mar. 63:71–77.

[5] See Fennessy, 2006. (In *literature cited* section.)

[6] See Reñones et al., 2007.

[7] See Seyboth et al., 2011.

trinsic feature of the studied population rather than a sampling effect related to emigration toward deeper offshore habitats.

Dusky groupers from Carpinteiro Bank were older (mean age=7.4 years; maximum observed age=40 years) than fish from the inshore rocky jetties located in the mouth of Patos Lagoon in Rio Grande (mean age=5.0 years; maximum observed age=12 years; Seyboth et al., 2011). Our study provides compelling evidence that younger (<7 years) dusky groupers from Carpinteiro Bank are also significantly larger than similar-age fish from the rocky jetties. Specifically, the sizes of offshore groupers at ages 3–6 are 11–19% larger than inshore dusky groupers—a finding that leads to the hypothesis that environmental quality at the deeper offshore habitat may be better than that at the shallow inshore habitats.

In this study, we assume that differences on mean size-at-age between fish from the inshore and offshore habitats may be associated with differences in environmental quality between both sites, and several types of evidence support this hypothesis. For example, freshwater runoff from the Patos Lagoon estuary is, perhaps, the most important physical factor that influences the studied population, particularly during El Niño events, when freshwater discharge into the lagoon increases and pushes the estuarine plume over the inshore zone (Garcia et al., 2003), decreasing salinity and disturbing fish communities (Garcia et al., 2003; Garcia et al., 2004). The large variations in salinity periodically experienced by the inshore population may cause osmotic stress, increasing the energy outlay required to maintain fish homeostasis (Schmidt-Nielsen, 2002). In contrast, the salinity variation at the offshore bank usually ranges from 26.8 to 36.6 (Moller[2]), a level that is roughly a third of the salinity variability measured

[2] Moller, O. 2012. Unpubl. data. Laboratório de Oceanografia Costeira e Estuarina, Institute de Oceanografia, Universidade Federal de Rio Grande, CP. 474, 96200-900, Brazil.

at the inshore habitat (10.6 and 35.1 during winter and summer, respectively) (A. Garcia, unpubl. data). In fact, faster growth was reported in dusky groupers reared under a salinity of 35 compared with dusky groupers reared under mixohaline treatments with salinities of 20 and 27 (Gracia-López and Castelló-Orvay, 2003), indicating that less energy may be expended for body homeostasis in more salinity-stable marine waters than in more salinity-variable estuarine conditions. This evidence supports the hypothesis that salinity regimes may play a significant role in controlling the differential growth rates of dusky groupers observed in inshore and offshore habitats.

An additional environmental factor capable of influencing fish growth in our system is food availability. The diet of groupers from the rocky jetties of Rio Grande consisted mostly of small crabs (Condini et al., 2011), whereas groupers from the Carpinteiro Bank were found to feed mostly on fish (Condini, in press). Given that fish are known to be a higher-energy food resource than crustaceans (Reñones et al., 2002), food quality also may partially explain the faster growth observed in dusky groupers sampled at the Carpinteiro Bank.

Conclusions

Accurate age and growth parameters of fish populations are important for the efficiency of environmental protection actions. In this study, we have shown that dusky groupers from their southernmost population present growth performance similar to that observed worldwide, with maximum size and age of about 900 mm TL and 40 years, respectively. We have also shown that younger individuals with ages between 3 and 6 years sampled offshore (depths of 15–25 m) are larger on average than fish of the same age sampled inshore (depths <5 m). These findings indicate that offshore banks may provide higher quality habitats for dusky groupers than do inshore rocky habitats. Therefore, the dusky grouper population that inhabits this offshore bank should be a preferable target for fishery management actions (e.g., catch monitoring and fishing quotas) to prevent increases in the level of overfishing of this endangered species at its southernmost distribution limit in southwestern Atlantic.

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Abstract—Twenty homologous microsatellite markers, or simple sequence repeats (SSRs), were developed for blackfin tuna (*Thunnus atlanticus*) through the use of a direct *Seq-to-SSR* approach. The number of alleles per locus ranged between 5 and 26, and the expected heterozygosity ranged between 0.640 and 0.969. Three loci displayed significant departure from Hardy-Weinberg equilibrium expectations, likely reflecting occurrence of null alleles. Another locus showed consecutive alleles that differed by one base pair only. Consequently, this locus may be prone to elevated rates of scoring errors. The remaining 16 loci will be valuable for studies in conservation genetics of blackfin tuna.

Development and characterization of microsatellite markers for blackfin tuna (*Thunnus atlanticus*) with the use of Illumina paired-end sequencing

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The blackfin tuna (*Thunnus atlanticus*) is a small tuna widely distributed in tropical and subtropical waters of the western Atlantic Ocean from the mid-Atlantic region of the East Coast of the United States south to northern Brazil (Collette and Nauen, 1983). Although this species is exploited by fisheries in several countries, management of this resource in the United States and abroad is almost inexistent and, in particular, it is not known to date whether multiple stocks of this species occur within its recorded distribution range (Mathieu et al., 2013).

Tagging studies conducted in the island nations of St. Vincent and the Grenadines and Bermuda have indicated that blackfin tuna exhibit some degree of site fidelity for a significant proportion of tagged fish that have been recaptured in close proximity of their tagging location, sometimes over multiple years (Luckhurst et al., 2001; Singh-Renton and Renton, 2007). However, long distance movements also were reported for some of the individuals tagged in the study of Singh-Renton and Renton (2007). In such a situation, the analysis of genetic variation at molecular markers is expected to provide valuable information on the structure of stocks to develop sustainable management of fisheries (Carvalho and Hauser, 1994). Microsatellites are the most widely used markers for genetic studies of exploited fishes; however to date homologous markers for the blackfin tuna are not available.

The objective of this work was to develop homologous microsatellite markers, or simple sequence repeats (SSRs), for studies in conservation genetics of this species. The application of next-generation sequencing technologies greatly enhances microsatellite discovery through direct screening of short sequences of genomic DNA, hereafter *reads*, for repeat arrays without the need for cost-prohibitive steps, such as cloning of genomic libraries and screening of individual clones through Sanger sequencing. The *Seq-to-SSR* method of Castoe et al. (2012) is based on direct screening of unassembled sequencing reads, an approach that further increases the cost effectiveness of the

procedure. In our study, this method was applied to rapidly identify microsatellite loci in the blackfin tuna.

Materials and methods

Genomic DNA from fin tissue of one blackfin tuna specimen collected offshore of the Louisiana coastline was extracted by using a standard phenol-chloroform protocol (Sambrook et al., 1989). An Illumina[1] paired-end library, which allows sequencing of both ends of DNA fragments, was prepared and sequenced on an Illumina HiSeq 2000 platform (Illumina, San Diego) according to methods described by Castoe et al. (2012). Reads were quality controlled and trimmed for low-quality data (phred scores <30). In the program PAL_FINDER, vers. 0.02.03, a Perl script developed by Castoe et al. [2012] and available at http://sourceforge.net/projects/palfinder/), 5,874,294 reads were screened for microsatellite arrays that contained a minimum of 12 repeats. Use of a minimum of 12 repeats, in our experience, ensures that the selected microsatellite loci are likely to be polymorphic. The search in PAL_FINDER was continued until 45 dinucleotide loci and 5 tetranucleotide loci were discovered, requiring screening of 286,240 reads and the whole database (5,874,294 reads) for dinucleotides and tetranucleotides, respectively.

Specific polymerase chain reaction (PCR) primers were designed with the open-source software Primer3 (Untergasser et al., 2012; Koressaar and Remm, 2007; source code available at http://sourceforge.net/projects/primer3/). The designed primers were tested for amplification success and scoring reliability and then evaluated at different annealing temperatures using blackfin tuna samples provided by the Louisiana Department of Wildlife and Fisheries. Samples had been collected offshore of the Louisiana coastline during the spring of 2013. Amplification success of the candidate loci during PCR was tested by assaying 6 specimens. PCR reactions were performed in a total volume of 5.6 µL that contained 7–13 ng of genomic DNA, 2.2 pmol of each primer, 1.1 nmol of dNTPs (Promega Corp., Madison, WI), 8.4 nmol of $MgCl_2$ (Promega), 0.28 U of Taq polymerase (Promega), and 1X of buffer (Promega). Amplification by PCR consisted of an initial denaturation at 95°C for 5 min, 35 cycles of 95°C for 30 s, annealing temperature (T_a) for 30 s, 72°C for 45 s, and a final extension of 15 min at 72°C. T_a was 62°C for the first 7 cycles, 60°C for the following 7 cycles, and 56°C for the remaining 21 cycles. The obtained PCR products were evaluated through electrophoresis on high-resolution NuSieve GTG Agarose gels (Lonza Group, Basel, Switzerland).

Loci that showed consistent amplification success and polymorphic PCR products were selected for further testing, and the 5′ end of 1 of the 2 primers was labeled by using one of the fluorescent dyes 6-Fam, Hex, or Ned to allow detection and scoring on an automated DNA sequencer. Evaluation of scoring reliability of the tested loci was based on DNA samples from 8 blackfin tuna specimens. The optimal T_a for amplification of each locus was then determined during PCR reactions by using DNA from 4 blackfin tunas, and was carried out at 6 annealing temperatures held constant through the 35 amplification cycles and ranging from 52°C to 62°C. PCR products were run on an ABI Prism 377 96-lane DNA Sequencer (Applied Biosystems, Life Technologies, Carlsbad, CA). The obtained electropherograms were analyzed with Applied Biosystems GeneScan software, vers. 3.1.2 (Life Technologies), and alleles were scored in Applied Biosystems Genotyper software, vers. 2.5 (Life Technologies). The polymorphic loci that could be scored reliably were characterized statistically on the basis of the genotypes of 35 blackfin tuna specimens provided by C. Pau and L. Reynal, both of IFREMER, La Martinique. Specimens had been caught offshore of the island of Martinique in the summer of 2013.

Results

Amplification tests were conducted on 50 primer pairs, 45 potentially amplifying dinucleotide microsatellites, and 5 amplifying tetranucleotide microsatellites. Twenty-four loci were amplified consistently across the tested specimens and were all labeled with fluorescent dyes for further evaluation and optimization of annealing temperature. Twenty loci (19 dinucleotides and 1 tetranucleotide) gave scorable PCR products and are described in Table 1. The number of alleles (A), expected heterozygosity (H_e), and inbreeding coefficient (F_{IS}) were calculated with the software FSTAT, vers. 2.9.3.2 (Goudet, 1995). Per locus estimates ranged from 5 to 26 for A, from 0.640 to 0.969 for H_e, and from −0.003 to 0.268 for F_{IS}. Conformance of genotypic proportions to Hardy-Weinberg (H-W) equilibrium expectations was tested with exact tests in the software GENEPOP, vers. 4.1 (Raymond and Rousset, 1995; Rousset, 2008). Genotypic proportions did not depart significantly from H-W equilibrium expectations, except for the loci BT6, BT27, BT47, and BT91. The departure at locus BT27 was not significant after Bonferroni correction (Rice, 1989). Analyses in Micro-Checker, vers. 2.2.3 (Van Oosterhout et al., 2004) revealed possible occurrence of null alleles at loci BT6, BT47, and BT91. There was no evidence of scoring artifacts at locus BC27.

Discussion

On the basis of the significant departure of genotypic proportions from Hardy-Weinberg equilibrium expectations and the inference of null alleles at loci BT6,

[1] Mention of trade names or commercial companies is for identification purposes only and does not imply endorsement by the National Marine Fisheries Service, NOAA.

Table 1

Summary data for 20 microsatellites developed for the blackfin tuna (*Thunnus atlanticus*). T_a: annealing temperature; A: number of alleles; H_e: expected heterozygosity; F_{IS}: inbreeding coefficient; $P_{H\text{-}W}$: probability of conformance to Hardy-Weinberg (H-W) equilibrium expectations. Values in bold represent significant departures from H-W expectations after Bonferroni correction. An asterisk (*) indicates that the sequence of the microsatellite array could not be determined in its entirety because of insufficient overlap of the 2 paired-end reads (the reported number is the highest number of repeats contained within 1 of the 2 paired-end readings).

Locus	Repeat motif	Primer sequence (5′–3′) label	T_a (°C)	A	Allele size range (base pairs)	H_e	F_{IS}	$P_{H\text{-}W}$
BT4	$(CA)_2TA(CA)_{13}$	F: GGTTGAAGTGCTTCAGGTGTAA (FAM) R: CCTGCCACAGAATGTCTGAA	58	17	134–176	0.905	0.038	0.0972
BT6	$(CA)_{17}$ *	F: ACAGCAGACTGTCAAACACAAA (HEX) R: CTTGTGGCAGAAGCTCTTGA	62	30	142–242	0.969	0.145	**0.0006**
BT11	$(CA)_{13}$ *	F: AACCTTAACCACTGACCCAAA (FAM) R: CTGGTTAGAGCTTTGTCATTGC	62	22	198–258	0.949	0.039	0.8843
BT18	$(CA)_{12}$	F: CCATAAAACACTGCCTGTCAGA (HEX) R: AGAAACGCTTGGATGCAACT	54	15	96–136	0.923	-0.017	0.2059
BT20	$(CA)_{14}TA(CA)_4$ *	F: GCGAGACTTGTGCCTGACTT (HEX) R: ATGGCATGGTAGGTGATGGT	62	20	172–224	0.929	0.05	0.2354
BT22	$(CA)_{18}$ *	F: TATCTGATAAGCAGGGAAGACC (HEX) R: AAATTTGCAGTGCGTCCTAA	56	18	163–243	0.903	0.055	0.1326
BT27	$(CA)_{17}$	F: CCACATTACAATCCGTGATCC R: GCGTTTAGGTAACTGCTGGTGT (FAM)	62	14	233–273	0.89	0.052	0.0197
BT29	$(CA)_{14}$ *	F: CACGACAGGCCTAATCAATC R: TTGAGGCCTACAGATGTCAGA (NED)	62	19	162–208	0.915	0.001	0.5591
BT31	$(CA)_{25}$ *	F: CATGACCCAGGAAACACCTT R: AAATGCCCTTGATGCTGTATG (FAM)	58	12	365–403	0.89	0.042	0.2107
BT37	$CACC(CA)_{12}$	F: TGATCAGCTATGAACGGTCCTT (HEX) R: GTGCTTGCTCCTGAAGCTCT	62	5	304–316	0.646	0.16	0.0503
BT47	$(GA)_{15}$	F: GGATGGTGCACAGGTTCTC (FAM) R: CAGGAACATCAACAGGAATGAA	54	13	126–156	0.776	0.263	**0.0008**
BT67	$(CA)_{12}$	F: CAGGCTGAGGATGGTGCTAC R: TGTATCACACCGCCAGGTAA (FAM)	62	8	137–163	0.67	-0.086	0.108
BT68	$(CA)_{13}$	F: GTAAGCACCAAGCGTGTGAA (HEX) R: AGCAGGCTGAGCTTAACTGG	62	10	95–113	0.815	-0.003	0.75
BT71	$(CA)_3CG(CA)_{20}$	F: TTAACGAGGCTGTACTTGCTCA R: GTGAGGGTCACGCTTTATGC (HEX)	62	26	225–305	0.943	0.061	0.0698
BT73	$(CA)_{26}$	F: GCCTGCTACTCCGAGGTAA (FAM) R: TGTTGTTCTGTAAACCCAGCTT	62	25	229–387	0.952	0.011	0.5812
BT81	$(CA)_{13}TACA$	F: AGGCTCAAGGGTCCACATC (FAM) R: GGGCCAAGCTGAACAGTAAA	62	12	210–232	0.852	0.137	0.3411
BT83	$(CA)_{13}$	F: AGGCCTGACTACAAATCTGTT (HEX) R: CAGTGGTGAGTAACAGCATGTC	62	7	144–158	0.64	-0.16	0.898
BT88	$(CA)_{19}$ *	F: AGCTTGGTGTAATTGCAAAGAC (FAM) R: AGGAGCAGATGCTCTCAGGA	58	19	151–197	0.924	-0.051	0.6784
BT91	$(GATA)_{12}$ *	F: CAGACCCTTAGTGGCTTGCT (NED) R: CGCTTCCAGGGTTAGTTGTT	62	20	155–211	0.937	0.268	**0.0005**
BT95	$(CA)_{12}$ *	F: ATTGTGCAAAGGGAGGTGTT R: TCTTTCTCGTGACCGATGTG (HEX)	58	14	190–234	0.897	0.013	0.7854

BT47, and BT91, we caution against the use of these 3 microsatellites for population genetic studies of the blackfin tuna. Genotypic frequencies at locus BT27 did not depart significantly from Hardy-Weinberg equilibrium expectations, but analyses in Micro-Checker indicated that null alleles may occur at this microsatellite. This marker, therefore, will need to be evaluated further with larger sample sizes to determine its suitability for studies of population genetics of the blackfin tuna. Finally, locus BT71 showed occurrence of rare shifts of one base pair (i.e., consecutive alleles differed by only one base pair, departing from the expected pattern of variation at microsatellites). Because of the limited resolution of acrylamide gels, the ability to reliably score alleles that differ by one base pair is challenging and, consequently, this locus may be prone to elevated rates of scoring errors. The microsatellite markers developed during our study will be available for conducting studies of the genetic stock structure of blackfin tuna, investigations that are needed to assist in the design of management plans for the sustainability of this species.

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Southern flounder (*Paralichthys lethostigma*) stock structure inferred from otolith shape analysis

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Abstract—Examination of otolith morphometric variation has been shown to provide improved descriptions of stock structure for several marine fish species. We examined spatial variation in otolith shape of southern flounder (*Paralichthys lethostigma*) to understand population structure at the following geographic levels: ocean basin (Atlantic and Gulf of Mexico); regional coastal waters (Texas, Florida) and (Georgia, South Carolina); and local coastal waters (North Carolina). To reduce variability, we considered only age-1 female fish. From digitized otolith images, we extracted descriptions for common shape indices and elliptical Fourier coefficients and found strong evidence for differences at the ocean basin scale, but only weak evidence for structure at either within-basin (i.e., among states) or within-state (local) spatial scales. Our finding of inferred stock structure differences between the ocean basins aligns with the geographic break in the distribution of this species—the absence of this species from the southern portion of Florida—as well as with recent genetic findings. Currently, state-level management of southern flounder in both areas does not account for any basin-wide population mixing and, therefore, by default, assumes a separate unit stock for each state, although our findings indicate that mixing could be extensive. Additional sources of information (e.g., genetics, life history traits) collected at appropriate spatial scales should be examined to confirm suspected levels of mixing and to determine suitable management strategies for the conservation of southern flounder stocks throughout their ranges.

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Although many marine populations once were considered to be panmictic on the basis of large geographical ranges and larval dispersal over long distances, results from improved stock identification methods (e.g., mtDNA, parasite community, and shape analyses) are calling into question some initial assumptions of population homogeneity (Cadrin et al., 2005). For example, although only very small amounts of gene flow may be required to homogenize considerable genetic variation without selection (Palumbi, 2003), evidence is accumulating from multiple species to support the existence of fine-scale geographic structure in several adaptive traits (Conover et al., 2006).

Regardless of their genetic similarities or differences, fish stocks possessing variable traits that can affect their responses to harvest still must be delineated clearly to achieve management objectives related to yield maximization and biomass conservation (Ricker, 1958; Begg et al., 1999; Conover et al., 2006). Spatial structure of fish stocks and the allocation of fishing effort, therefore, should be considerations in the management of any species (Stephenson, 1999; Ying et al., 2011) because ignoring population structure can lead to negative outcomes, such as loss of genetic diversity and reduction in the yield-generating potential of a stock (Pawson and Jennings, 1996; Bailey, 1997; Booke, 1999). Within fisheries science, stock definitions vary but are focused largely on consistency of unique traits—the characteristics that distinguish a stock should remain constant through time and be unique to that stock (Ihssen et al., 1981; Booke, 1999) for both conservation and harvest purposes (Cadrin, 2000).

Methods of phenotypic stock identification have expanded greatly from abundance and meristic approaches to now include the use of both natural and artificial tags, examination of life history traits, population genetics, and morphometric outlines (reviewed in Cadrin et al., 2005). Recently, the study of closed-form structures, such as otoliths and scales, has increased with the advent of computers that are able to rapidly analyze large amounts of data. In addition, otoliths are collected rou-

tinely for age determination purposes, making large sample sizes available for shape analyses. Consequently, numerous examples of successful discrimination of fish stocks based on otolith morphometrics and shape descriptors exist.

Campana and Casselman (1993) were among the first to use otolith shape as an indicator of stock variation. They conducted an exhaustive study of all 3 types of otolith pairs in which they found evidence of structuring among spawning groups of Atlantic cod (*Gadus morhua*) in the Northwest Atlantic, in addition to differences in otolith shape among age groups, sexes, and year classes. Begg and Brown (2000) used otolith shapes to challenge successfully the assumption of a single stock of haddock (*Melanogrammus aeglefinus*) at Georges Bank, and DeVries et al. (2002) clarified previous tag and genetic data when they used otoliths to successfully distinguish stocks of king mackerel (*Scomberomorus cavalla*) from the Gulf of Mexico and the Atlantic that were sampled during their winter mixing off southern Florida. More recently, otolith shape analysis has been done at varying spatial scales for dolphinfish (*Coryphaena hippurus* [Duarte-Neto et al., 2008]), North Atlantic saury (*Scomberesox saurus saurus* [Agüera and Brophy, 2011]), and anglerfish (*Lophius piscatorius* [Cañas et al., 2012]) to help clarify questions about geographic population structure.

The southern flounder (*Paralichthys lethostigma*) occurs in the Northwest Atlantic and Gulf of Mexico from North Carolina to Texas; however, this species does not occur around the southern tip of the Florida peninsula (Gilbert[1]). Southern flounder in the South Atlantic and Gulf of Mexico basins are considered separate genetic stocks (Anderson et al., 2012). Management for the range of this species occurs generally at the individual state level, despite a high likelihood of within-basin mixing during offshore spawning migrations of adults and the possibility of year-round offshore residents (Watterson and Alexander[2]; Taylor et al.[3]).

Southern flounder support important commercial and recreational fisheries throughout their range, with females contributing most to the landings because growth is greater in females than in males. In 1990–2010, more than 30,000 metric tons were landed commercially, and the vast majority (~98%) of these landings took place in North Carolina. Over the same period, recreational landings were about 50% lower in magnitude and were more evenly distributed among states between the Gulf of Mexico and South Atlantic basins (NMFS[4]). However, recreational harvest may be a primary factor that is contributing to population declines in the Gulf of Mexico (Froeschke et al., 2011), and these declines have prompted a new stock enhancement program in Texas aimed at supplementing natural reproduction.

Because no directed fishery exists for southern flounder in offshore habitats and exchange of individuals among states is not well understood, state management agencies assume unit stocks on the basis of state boundaries. However, population structure that does not coincide with state boundaries has been shown with other flounders in the Northwest Atlantic that share geographic ranges of a similar size and life history characteristics with the southern flounder. These flounders include the southern flounder congener summer flounder (*P. dentatus* [Burke et al., 2000]), winter flounder (*Pseudopleuronectes americanus* [DeCelles and Cadrin, 2011]), and yellowtail flounder (*Limanda ferruginea* [Cadrin, 2010]).

Interestingly, each of these studies reported stock structuring at varying scales. Summer flounder were found to have structure related to the biogeographic boundary of Cape Hatteras in North Carolina, whereas evidence indicated that winter flounder and yellowtail flounder had population structures of much finer scales, including the existence of up to 3 stocks within New England waters. Yet despite evidence for within-basin genetic homogeneity (Anderson et al., 2012), flounder residency within specific estuaries for the first few years of life may create regional phenotypic differences that reflect local adaptation. For example, estimates of the von Bertalanffy growth coefficient (K) for female southern flounder vary considerably among states. For fish in Texas, Stunz et al. (2000) estimated K at 0.75, and Fischer and Thompson (2004) estimated K at 0.51 for Louisiana fish. Within the South Atlantic basin, estimates of K have been lower: 0.23 for fish in South Carolina (Wenner et al.[5]) and 0.28 for fish in North Carolina (Takade-Heumacher and Batsavage[6]).

[1] Gilbert, C. R. 1986. Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (South Florida)—southern, gulf, and summer flounders. U.S. Fish Wildl. Serv. Biol. Rep. 82 (11.54). U.S. Army Corps Eng. Tech. Rep. TR EL-82-4, 27 p. [Available from http://www.nwrc.usgs.gov/wdb/pub/species_profiles/82_11-054.pdf.]

[2] Watterson, J., and J. Alexander. 2004. Southern flounder escapement in North Carolina. Final performance report F-73, Segments 1–3, 41 p. [Available from North Carolina Department of Environment and Natural Resources, Division of Marine Fisheries, P.O. Box 769, Morehead City, NC 28557.]

[3] Taylor, J. C., J. M. Miller, and D. Hilton. 2008. Inferring southern flounder migration from otolith microchemistry. Final report for Fishery Resource Grant 05-FEG-06, 27 p. [Available from North Carolina Sea Grant, NC State Univ., Campus Box 8605, Raleigh, N.C. 27695-8605.]

[4] NMFS (National Marine Fisheries Service). 2011. Personal commun. Fisheries Statistics Division, NMFS, Silver Spring, MD 20910.

[5] Wenner, C. A., W. A. Roumillat, J. E. Moran Jr., M. B. Maddox, L. B. Daniel III, and J. W. Smith. 1990. Investigations on the life history and population dynamics of marine recreational fishes in South Carolina: Part 1. Final report for project F-37, 187 p. Marine Resources Research Institute, South Carolina Wildlife and Marine Resources Department. Charleston, SC. [Available from the Marine Resources Research Institute, South Carolina Department of Natural Resources, 217 Fort Johnson Rd., Charleston, SC 29412.]

[6] Takade-Heumacher, H., and C. Batsavage. 2009. Stock status of North Carolina southern flounder (Paralichthys lethostigma), 91 p. North Carolina Division of Marine Fisheries, Morehead City, NC. [Available from http://00de17f.netsolhost.com/fmps/downloads/souflounderSA.pdf.]

Mean sizes at age have also varied among these studies—mean sizes at age-1 were 288 mm in total length (TL) in South Carolina and 396 mm TL in Louisiana (Wenner et al.[5]; Fischer and Thompson, 2004). Emergent patterns from tagging studies of southern flounder also provide evidence that supports the possibility of phenotypically distinct stocks. Several tagging studies initiated in North Carolina waters have arrived at the same 2 general conclusions. First, the majority of tagged southern flounder were recaptured very close to the tag site (although this result could be more of a reflection of sampling effort than of actual movement and distribution). Second, those fish that were captured away (>20 km) from the tag site were collected in locations exclusively south of the release location (Monaghan[7]; Craig and Rice[8]).

These studies indicate that at least among younger age classes (i.e., age-1 and age-2 fish that dominate commercial and recreational landings), southern flounder have restricted home ranges and may be isolated geographically from other stocks. Such site fidelity to certain locations and subsequent environmental conditions could contribute to phenotypic differences. Additionally, the documented migration south may occur over considerable distances (individuals tagged in North Carolina have been recaptured in Florida), and even small numbers of migrating individuals could suffice to genetically homogenize basin populations (Palumbi, 2003).

Genetic differentiation of southern flounder at the basin level has been established previously (Anderson et al., 2012), and our objective was to examine variation in otolith shape throughout the range of this species—in the South Atlantic, in particular— to identify possible phenotypic stocks at the within-basin scale. Although other methods of phenotypic stock identification (e.g., testing for spatial variation in growth rates or meristics) are also useful, powerful statistical methods to evaluate variation in otolith shape have developed rapidly in recent years (Stransky, 2013). Further, some of the variation in otolith shape is genetically determined, and therefore such variation should be comparatively less sensitive to short-term changes in environmental conditions. Three spatial scales were examined for possible population structure—between-basins (Gulf of Mexico and South Atlantic), within-basin (among sites within the South Atlantic and within the Gulf of Mexico), and within-state (among sites within North Carolina). Any descriptions of geographic stocks (or lack of) will be useful both in current management of the stocks and for the design of studies to examine differences in stock production that could inform future regional management of the southern flounder.

Materials and methods

Data collection

Southern flounder were collected in the South Atlantic from state waters (≤3 nautical miles, or 5.6 km, from the coasts) of North Carolina, South Carolina, Georgia, and Florida (Fig. 1) and were identified by morphological features described in a U.S. Fish and Wildlife species profile (Gilbert[1]). In the analysis for this study, 289 right otoliths from age-1 southern flounders were used. Sixty-five samples were collected from the Gulf of Mexico, and the remaining 224 came from the Atlantic states (Figs. 1 and 2). Because of low sample sizes from both Florida (Atlantic waters) and Georgia and the close proximity of the sampling locations from which fish were obtained in those 2 states (≤161 km between locations), Florida and Georgia samples were pooled to represent the southern extent of the range in the U.S. South Atlantic. Additionally, where available, samples from the Gulf of Mexico (locations off both Florida and Texas) were included to enable between-basin comparisons.

Given the previously documented genetic differences (Anderson et al., 2012), we expected to also detect sufficient contrast in otolith shape of southern flounder between the basins that would aid our interpretation of variation at finer spatial scales. We explored 3 levels of spatial resolution for possible stock differentiation. The between-basin scale was explored to compare differences in otolith shape with established genetic differences. Within each basin, we examined variation among states because state boundaries delineate current "stocks" for management of southern flounder. We examined variation between 2 states within the Gulf of Mexico and 3 states in the U.S. South Atlantic. Finally, we investigated the possible existence of shape differences at a local spatial scale by examining fish from 3 distinct areas within North Carolina (Fig. 2), from which it is unlikely that juveniles would have moved.

Fish were collected in North Carolina during the fall of 2009 and 2010 as part of the North Carolina Division of Marine Fisheries (NCDMF) fishery-independent gillnet sampling program. Additional samples were purchased from licensed seafood dealers and were obtained through participation in directed commercial trips. Samples from South Carolina, Georgia, and Florida were collected during the fall of 2010 and 2011 as part of existing fishery-independent sampling programs in each state. Additional samples were obtained from

[7] Monaghan, J. P., Jr. 1996. Migration of paralichthid flounders tagged in North Carolina. Study 2. *In* Life history aspects of selected marine recreational fishes in North Carolina. Completion Report Grant F-43, Segments 1–5, p. 2.1–2.44. North Carolina Department of Environment and Natural Resources, Division of Marine Fisheries, P.O. Box 769, Morehead City, NC.

[8] Craig, J. K., and J. A. Rice. 2008. Estuarine residency, movements, and exploitation of southern flounder (Paralichthys lethostigma) in North Carolina. Final Report Fishery Resource Grant 05-FEG-15, 39 p. [Available from North Carolina Sea Grant, NC State Univ., Campus Box 8605, Raleigh, NC 27695-8605.]

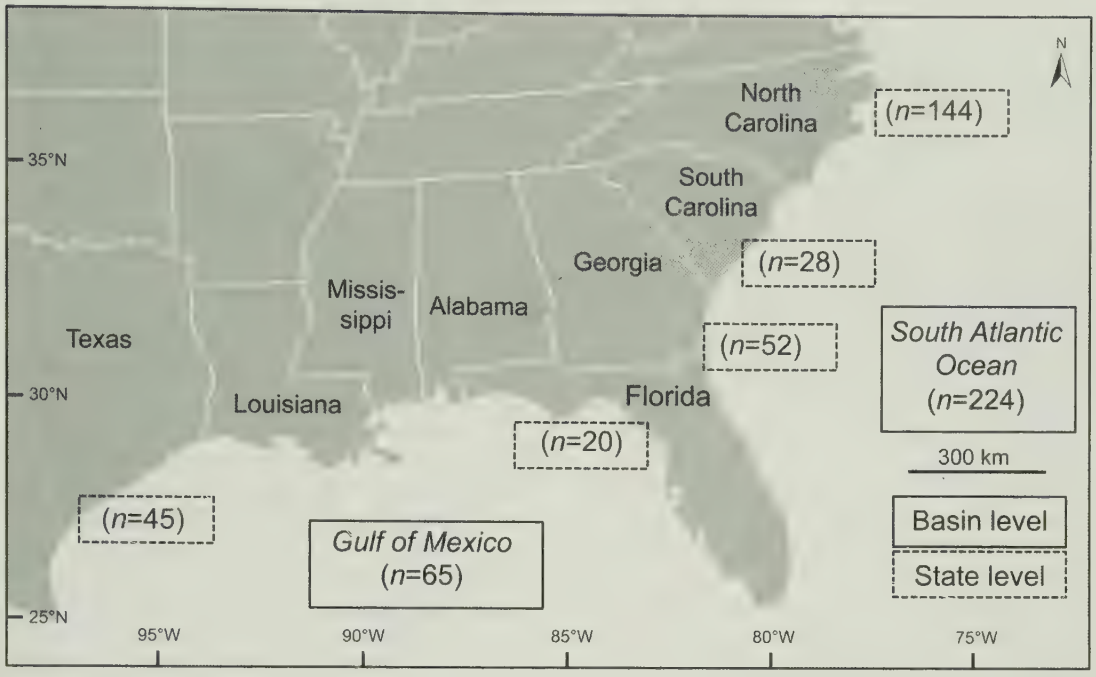

Figure 1

Map with sample sizes by state and basin for collections of age-1 female southern flounder (*Paralichthys lethostigma*) used in this study of the population structure of this species in the South Atlantic and Gulf of Mexico. Sample collections occurred in state waters in these 2 basins, the South Atlantic (North Carolina, South Carolina, Georgia, and Florida) and the Gulf of Mexico (Florida, Alabama, Mississippi, Louisiana, and Texas) in 1996 and in 2009–12 as part of state or federal agency sampling programs and from seafood dealers. Basin sample sizes are the sum of state sample sizes within each respective basin. Although we collected fish from both Georgia and Florida, in order to increase sample size, the samples from these 2 states were combined on the basis of their close geographic proximity.

Florida during the fall of 2012 through purchases from licensed seafood dealers and participation in directed commercial trips. Southern flounder were collected from the Gulf of Mexico in the fall of 1996 from inshore and offshore locations in the vicinity of Panama City, Florida, as part of sampling conducted by the NOAA Southeast Fisheries Science Center. Additional Gulf of Mexico samples were collected in Corpus Christi and Aransas bays, Texas, during 2009 and 2011 as part of a separate research program.

Because the age of fish and recent growth histories can have a considerable influence on otolith shape (Campana and Casselman, 1993), the analysis in this study focused on fish captured only during October, November, and December to reduce variability associated with fractional ages (although additional mathematical size corrections are detailed in the *Statistical procedure* subsection of the *Materials and methods* section). Furthermore, assuming a 1 January birthday for all fish, fall sampling meant that fish had completed the majority of their growth for the most recent year. Therefore, the translucent zone closest to the otolith edge was approaching maximum width and thus would allow confident annuli identification for aging purposes. Lastly, because the vast majority of individuals collected were age-1 females, our analysis of otolith shape focused on this sex–age group.

Otolith preparation and image analysis

Otoliths were extracted at the time of collection when additional data were also collected (e.g., TL in millimeters, mass in grams, and sex). After excision, otoliths were cleaned with isopropyl alcohol to remove any loose tissue, and then dried and stored in paper envelopes. All Atlantic-basin otoliths were aged whole with the NCDMF aging protocol (originally described in Wenner et al.[5]). Otoliths from the Gulf of Mexico had been aged previously, and those ages were provided for use in this study (Fitzhugh[9]; Nims[10]). Otoliths were first scanned on an Epson Perfection V500[11] photo scanner (Epson America Inc., Long Beach, CA) at high resolution (1200

[9] Fitzhugh, G. 2011. Personal commun. Panama City Lab, Southeast Fisheries Science Center, National Marine Fisheries Service, NOAA. Panama City, FL 32408.

[10] Nims, M. 2012. Personal commun. Univ. Texas Austin, Port Aransas, TX 78373.

[11] Mention of trade names or commercial companies is for identification purposes only and does not imply endorsement by the National Marine Fisheries Service, NOAA.

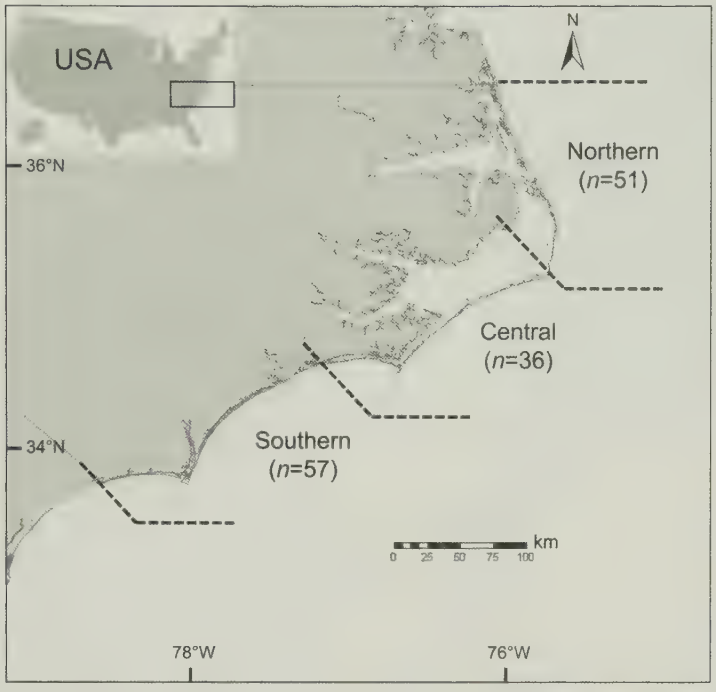

Figure 2

Map with sample sizes of age-1 female southern flounder (*Paralichthys lethostigma*) collected within the state waters of North Carolina in 2009 and 2010 as part of a sampling program of the North Carolina Division of Marine Fisheries (NCDMF) and from seafood dealers. The 3 regions are based on NCDMF sampling areas; however, they are characterized also by different estuaries. The northern region includes Albemarle Sound, the central region consists of the Neuse–Pamlico Estuary, and the southern region is defined by the Cape Fear and New River estuaries.

dots per inch), yielding images comparable in quality to those from high-resolution photographs. Use of a flatbed scanner permitted us to scan relatively large batches of samples simultaneously (without the bending error that is a concern with optical microscopy). Southern flounder otoliths are relatively flat, and all otoliths were scanned sulcus-side down in a uniform orientation.

Otolith shapes were described by using 1) elliptical Fourier analysis (EFA) and 2) calculation of univariate shape indices. Compared with other types of Fourier transforms (e.g., fast Fourier transform), EFA is thought to provide the most consistent results for this type of application (Mérigot et al., 2007). The EFA method decomposes the closed-form contour into separate x and y coordinates (Kuhl and Giardina, 1982). Generally, a predetermined number of harmonics is defined for analysis, and each harmonic involves 2 parametric functions that describe 4 coefficients (i.e., amplitudes), a_n, b_n, c_n, and d_n for the n^{th} harmonic.

In this study, elliptical Fourier coefficients (EFCs) were calculated with the program SHAPE, vers. 1.3 (Iwata and Ukai, 2002). SHAPE software extracts the chain-coded contour of each otolith (Freeman, 1974), and then the software normalizes (i.e., removes any size effects) the chain-code data on the basis of the first 3 coefficients of the first harmonics. As a result of this normalizing, the first 3 coefficients of the first harmonic are degraded ultimately and are unfit for analysis; the total number of coefficients = $(4\times H_n) - 3$, where H_n represents the number of harmonics investigated. This study extracted 10 harmonics, or 37 EFCs, for analysis.

Several size parameters and shape indices (Table 1) were also calculated for each otolith. Otolith area (in square millimeters) was provided as part of the SHAPE output analysis, and perimeter data (in millimeters) were calculated from the chain-code output file. Both otolith length and otolith width were measured (in millimeters) with ImageJ software, vers. 1.45 (National Institutes of Health, Bethesda, MD) according to otolith dimension definitions provided in Stevenson and Campana (1992). These size variables were then used to calculate several shape indices, including circularity, rectangularity, ellipticity, aspect ratio, and form function. Each of these variables is a common shape index used routinely in otolith morphometric investigations (Table 1; Russ, 1990).

Statistical procedures

Univariate shape indices were examined for normality through the use of normal quantile–quantile plots, and any non-normal distributions underwent log transformation in an attempt to satisfy the assumption of normality (Cadrin et al. 2005). To identify and control for the effect of otolith size on the shape indices, an analysis of covariance (ANCOVA) was used with geographic region as

Table 1

Size parameters, shape indices, and shape formulae used to describe otoliths from female southern flounder (*Paralichthys lethostigma*) collected in the U.S. South Atlantic and Gulf of Mexico in 1996 and in 2009–12 as part of state or federal agency sampling programs and from seafood dealers for this study of the population structure of southern flounder in these basins.

Size parameter	Shape index and formula
Area (A)	*Circularity* = P^2/A
Perimeter (P)	*Rectangularity* = $A/(OL\times OW)$
Otolith length (OL)	*Form function* = $4\pi A/P^2$
Otolith width (OW)	*Aspect ratio* = OL/OW
	Ellipticity = $(OL–OW)/(OL+OW)$

the factor and otolith length as the covariate. Both otolith length and fish TL have been used as covariates in similar analyses and were expected to yield similar results. However, Campana and Casselman (1993) recommended the use of otolith length because this measured variable is more robust to collection and preservation effects, in addition to its strong correlation with fish TL (in our study, correlation coefficient [r]=0.90). In the ANCOVA model, if the interaction of region and otolith length was significant (P<0.05), the shape index was excluded from the analysis because it could not be corrected (Tracey et al., 2006). When the interaction was not significant but the effect of otolith size was significant, the shape index was corrected through the use of the common within-group slope (b), whereby the product of the slope and otolith length was subtracted from the shape variable. One-way analysis of variance (ANOVA), followed by Tukey's honest significant difference (HSD) post-hoc comparisons and Welch's t-tests (with significance for both tests assessed at α=0.05), was used to examine differences in shape indices and to identify those indices that could be used in a discriminant analysis.

To identify the optimal number of harmonics for analysis, we ran cross-validation analyses to explore the descriptive power of harmonics. For this analysis, we started with the first 2 harmonics and subsequently added harmonics until the rate of jackknife reclassification success declined or plateaued, indicating that the additional harmonics no longer were increasing discriminatory power. Preliminarily, we evaluated separately 15 and 20 harmonics to examine the sensitivity of the analysis to the number of harmonics. With 15 and 20 harmonics, the mean (and standard deviation of the mean) reclassification success rate was nearly identical to or slightly worse—although not statistically significant—than the result from our original analysis with 10 harmonics, and, therefore, we limited our analysis to 10 harmonics. In addition to the statistical justification, we viewed 10 harmonics as a compromise between the parsimony of fewer harmonics indicated by cross validation and a larger number of harmonics based on the notion that fine-scale description is often contained in higher-order harmonics (Cadrin et al., 2005) and that cross validation might not detect these small differences.

Linear discriminant analyses were used to examine differences between geographical subsamples (i.e., putative stocks) at all 3 spatial scales. Ideally, the construction of discriminant functions for otolith shape benefits from the inclusion of both EFC and shape indices (Agüera and Brophy, 2011); therefore, any significant shape indices were considered for inclusion in the discriminant analysis.

Finally, jackknife reclassification (i.e., leave-one-out cross validation) was used to examine the classification success of the discriminant functions when classifying known-origin otoliths. Rates of reclassification success were compared with the null classification expectation (i.e., no structure) of $1/g$, where g was the number of groups or putative stocks in the analysis (White and Ruttenberg, 2007). Because unbalanced sample sizes can be problematic in discriminant function analysis (DFA) and result in a high rate of reclassification success by chance (White and Ruttenberg, 2007), we balanced our sample sizes on the basis of the smallest sample size in each analysis, and then we ran 1000 DFAs with all groups (except the smallest) randomized without replacement. We also conducted randomization tests of samples so that we would have not only a null point estimate but also a distribution (i.e., an expected range) to provide greater inference for our empirical results.

Results

Otolith shape indices

Circularity was the only otolith shape index that was not normally distributed, nor could it be normalized through transformation, and it was, therefore, dropped from the analysis of this study. With analysis of covariance, we detected no significant interactions between otolith length and location for any of the 4 remaining shape variables, which then were slope adjusted appropriately. At the basin level, all shape variables, except rectangularity, were significantly different (Table 2; Fig. 3). At the within-basin level, form function in the Gulf of Mexico was the only shape index that showed significant differences between states; no differences in shape indices were detected among Atlantic states. At a finer spatial scale (areas within North Carolina), differences in otolith shape indices were largely absent, with the exception of form function (Table 2).

Elliptical Fourier analysis

On the basis of the large number of EFCs (N=37) that were extracted in this study and a lack of high cumulative Fourier power (<70%, including all EFCs; Pothin et al., 2006), the descriptive power of harmonics was explored with analyses of cross validation. In the cross-validation analyses for each geographic scale, the rate of jackknife reclassification success plateaued almost immediately, indicating that each additional EFC provided minimal explanatory power. This result is in agreement with the finding of low Fourier power. Ultimately, all 4 discriminant analyses included the first 10 EFCs and any significant shape indices (Table 3).

Our basin-scale rate of reclassification success was nearly 80%, the highest level of reclassification success that we detected in any analysis and well outside the upper range of the null distribution (45–56%). Both within-basin reclassifications and the within–North Carolina reclassifications were marginally above—about a 6% improvement in classification—the range of the null expectation distribution (Table 3); however,

Table 2

Results, at 3 spatial scales, of hypothesis testing on univariate shape indices of otoliths from female southern flounder (*Paralichthys lethostigma*) that were collected in 1996 and in 2009–12 in state waters in 2 basins, the South Atlantic (North Carolina, South Carolina, Georgia, and Florida) and the Gulf of Mexico (Florida, Alabama, Mississippi, Louisiana, and Texas), as part of state or federal agency sampling programs and from seafood dealers.

Scale	Variable	Test statistic	*P*-value	Test statistic	*P*-value
Basin	Ellipticity	t=3.82	<0.001		
	Rectangularity	t=–1.67	0.100		
	Form function	t=–5.06	<0.001		
	Aspect ratio	t=3.74	<0.001		
		Within Gulf of Mexico		Within South Atlantic	
State	Ellipticity	t=1.48	0.145	F=1.71	0.184
	Rectangularity	t=1.48	0.149	F=0.20	0.815
	Form function	t=–2.06	0.046	F=0.23	0.797
	Aspect ratio	t=1.37	0.178	F=2.09	0.127
Region[1]	Ellipticity	F=0.55	0.581		
	Rectangularity	F=1.18	0.313		
	Form function	F=8.08	<0.001		
	Aspect ratio	F=0.51	0.600		

[1] Region within North Carolina. See text for further details.

the distribution of reclassification success rates overlapped with the null distribution of reclassification in each case (Fig. 4).

Discussion

Otolith shape and geographic distribution

Using a combination of univariate shape indices and EFCs, we found strong evidence for the existence of different populations of southern flounder between the 2 basins that we examined and less evidence for population structure as our spatial scope decreased (among states within each basin and among regions within North Carolina). Interestingly (and despite low samples sizes of fish from Florida waters in the Gulf of Mexico), neither of the within-basin analyses provided strong evidence of otolith shape differentiation, although the otoliths represented fish collected from the spatial extremes within each basin distribution. Both of these observations are in agreement with recent genetic findings for this species (Anderson et al., 2012). The use of EFA is considered one of the most reliable methods in otolith morphometric studies (Mérigot et al., 2007), and it took only 10 coefficients to produce a rate of reclassification success of nearly 80% at the basin level (Gulf of Mexico versus South Atlantic). Additionally, because of the resampling aspect of the DFA, this result should be considered particularly robust.

Of greater interest was a lack of evidence for stock differentiation at either the within-basin or within-state spatial scales. Although a number of factors could effectively mix individuals within each basin (or at finer spatial scales), tagging evidence does not support extensive mixing among or within states for younger, smaller fish, which typically remain within inshore estuarine systems (Monaghan[7]; Craig and Rice[8]). Although phenotypic stocks remain possible on the basis of previous observations of spatial variation in somatic growth rates (e.g., Stunz et al., 2000; Fischer and Thompson, 2004) and differences in otolith size estimates, our analysis of otolith shape did not clearly identify at the sub-basin level stocks of coastal southern flounder within either the South Atlantic or the Gulf of Mexico. We cannot rule out the possibility of undetected fine-scale structure; however, the strength with which otolith shape analyses are able to detect both large-scale genetic stock differences as well as fine-scale environmental differences indicates that within-basin structuring of southern flounder is likely weak.

The interpretation of variation in otolith shapes requires consideration of several factors that can contribute to otolith shape in the context of the species and study design. Otolith growth and shape are controlled dually by genetic and environmental influences (Vi-

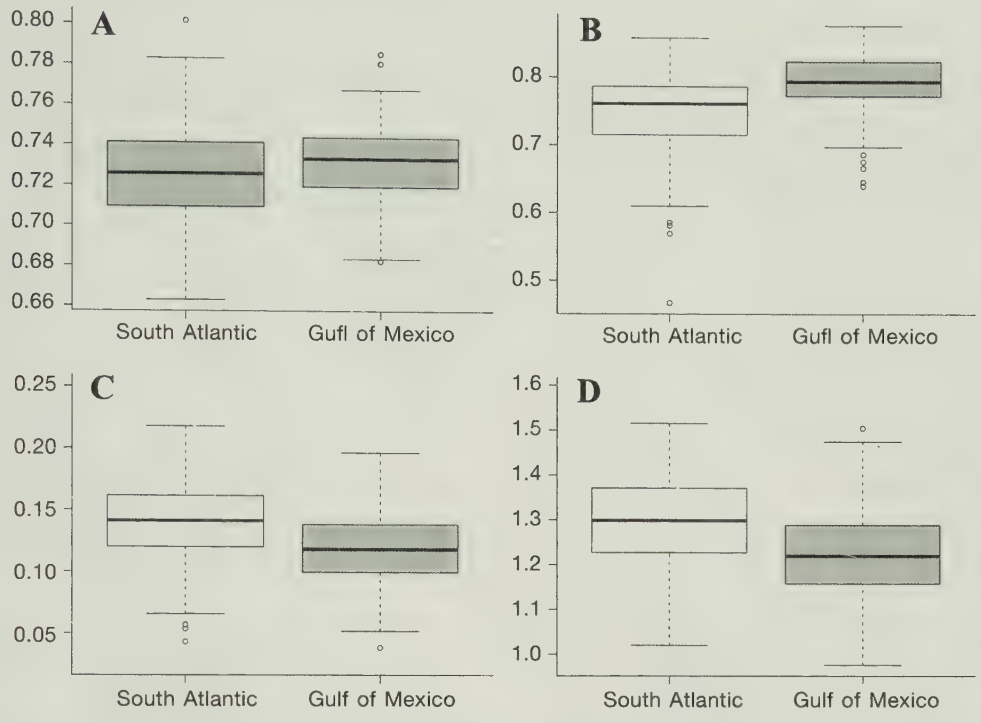

Figure 3

Boxplots of 4 shape indices for otoliths from female southern flounder (*Paralichthys lethostigma*) collected in 1996 and in 2009–12 in the U.S. South Atlantic and the Gulf of Mexico as part of state or federal agency sampling programs and from seafood dealers. Boxplots for the following shape indices are grouped by basin: (**A**) rectangularity, (**B**) form function, (**C**) ellipticity, and (**D**) aspect ratio. Significant difference (on the basis of Welch's *t*-test with α=0.05) between groups is indicated by different shadings of the boxes. Boxes represent the interquartile range; whiskers indicate the first and third quartiles +1.5 × interquartile range; and dots represent points beyond those defined by the whiskers.

gnon and Morat, 2010). Although the relative influence of these controls is the subject of ongoing research, early findings indicate that genetic influences determine the overall shape of an otolith and that environmental effects contribute finer morphological details (Hüssy, 2008; Vignon and Morat, 2010; Vignon, 2012).

Almost undoubtedly, southern flounder ranging from Texas to North Carolina experience a wide range of environmental conditions. Geographic gradients in environmental conditions may differentiate otolith shape sufficiently enough to enable detection of large-scale population structure, but there also may be considerable local environmental variation (e.g., salinity, temperature, and food) that effectively masks larger geographic patterns. Therefore, the ability to discriminate among even broadly spaced locales (e.g., North Carolina versus Florida) can be compromised.

Broad and fine-scale processes that affect coastal environments, which contribute to fish growth, and potentially otolith shape also can vary independently in time. One way to limit the influence of variable environmental effects is by controlling for year class, although significant year-class effects have been detected in some otolith shape studies (Castonguay et al., 1991) and not in others (Begg and Brown, 2000; Galley et al., 2006). Furthermore, in a study of orange roughy (*Hoplostethus atlanticus*), Gauldie and Crampton (2002) explored the idea of balancing selection—an alternating, generational morphology—operating to determine fish otolith morphology (which they observed on a 2-year cycle in their study). This balancing selection results in a persistent otolith polymorphism in populations that consist of multiple age classes. Polymorphism related to year-class effects could have contributed to within-group variation in our study because we included fish from multiple year classes, and polymorphism could have made it more difficult to detect broader regional differences in otolith shape.

Our study was improved by removal of possible sex effects, in addition to our collections being limited to relatively young (age-1) and mostly immature (Midway and Scharf, 2012) individuals. Because variable envi-

Table 3

Mean rate and range of jackknife reclassification success, standard error of the mean (SE), null expectations, and predictor variables associated with 3 discriminant function analyses that examined otoliths from female southern flounder (*Paralichthys lethostigma*) collected in 1996 and in 2009–2012 in the South Atlantic (North Carolina, South Carolina, Georgia, and Florida) and the Gulf of Mexico (Florida, Alabama, Mississippi, Louisiana, and Texas) as part of state or federal agency sampling programs and from seafood dealers. EFC=elliptical Fourier coefficients; g=number of groups.

	Basin	Within Atlantic	Within Gulf of Mexico	Within North Carolina
Mean reclassification success	0.79	0.47	0.66	0.47
Reclassification success range	0.74–0.84	0.38–0.56	0.53–0.77	0.39–0.55
SE	<0.01	<0.01	<0.01	<0.01
Expected mean ($1/g$)	0.50	0.33	0.50	0.33
Expected range	0.45–0.56	0.25–0.41	0.40–0.60	0.26–0.40
Predictor variables	Ellipticity Form function Aspect ratio 10 EFCs	10 EFCs	Form function 10 EFCs	Form function 10 EFCs

ronmental conditions and growth rates can be major determinants of changes in otolith shape (Campana and Casselman, 1993; Cardinale et al., 2004), we presumed that young southern flounder would most likely reflect the regionally different environments (if sufficient variability existed regionally) and, therefore, would make good candidates with which to detect the effects of those differences on their otolith shape. For instance, if spatial differences existed in growth conditions that were sufficient to generate distinct otolith shapes, then they should be greatest in younger fish at the end of the estuarine phase, when confidence in the spatial segregation of fish is high and the fish are growing rapidly while occupying habitats in which local conditions can cause variability in growth. Although variability in fine-scale processes, such as recent feeding history, has been shown to be an important determinant of otolith shape (Gagliano and McCormick, 2004; Hüssy, 2008), it was not practical for us to consider smaller spatial scales given the uncertainty in habitat use beyond the system of capture.

Considerable mixing of individuals from broadly separated areas during some phase of their life history also would dampen within-basin and within-state environmental effects on otolith shape. Once they mature, southern flounder emigrate from estuaries to participate in offshore spawning. Within the U.S. South Atlantic, individuals can migrate considerable distances (e.g., North Carolina fish recaptured in Florida; Monaghan[7]; Craig and Rice[8]), and therefore mixing of fish from different regions within the basin may be considerable. Therefore, despite the fact that, prior to maturity, many southern flounder likely occupy small home ranges in specific estuaries that span environmental gradients sufficient to generate differences in growth and otolith shape, a high degree of offshore mixing could result in a level of genetic homogenization that swamps local environmental effects.

One additional and less discussed factor that possibly affects otolith shape is time of capture. To minimize variation in body size, all of our fish were collected during late summer and fall. Each of the age-1 females that we used in this study, therefore, had the opportunity to take advantage of the warm summer growing season, a factor that helped to reduce size variability in our sample. However, this time of capture meant that our samples were taken near the end of a period of rapid otolith growth. In addition to the notion that otolith shape is less variable as fish age and mature (Campana and Casselman, 1993), Gauldie and Nelson (1990) also found that faster otolith growth (often occurring among the youngest age groups) resulted in long, thin aragonite crystals. Therefore, it is possible that periods of fast otolith growth can result in a wider variety of otolith shapes present within a stock, making distinctions less apparent among younger age groups. We were not able to address this possibility in our study because southern flounder of older ages are encountered much less frequently in estuarine waters than in offshore waters.

Implications for management

Our examination of variation of otolith shape to detect the population structure of southern flounder at 3 geographic scales has possible implications for management. The combination of established genetic differentiation (Anderson et al., 2012) and the high like-

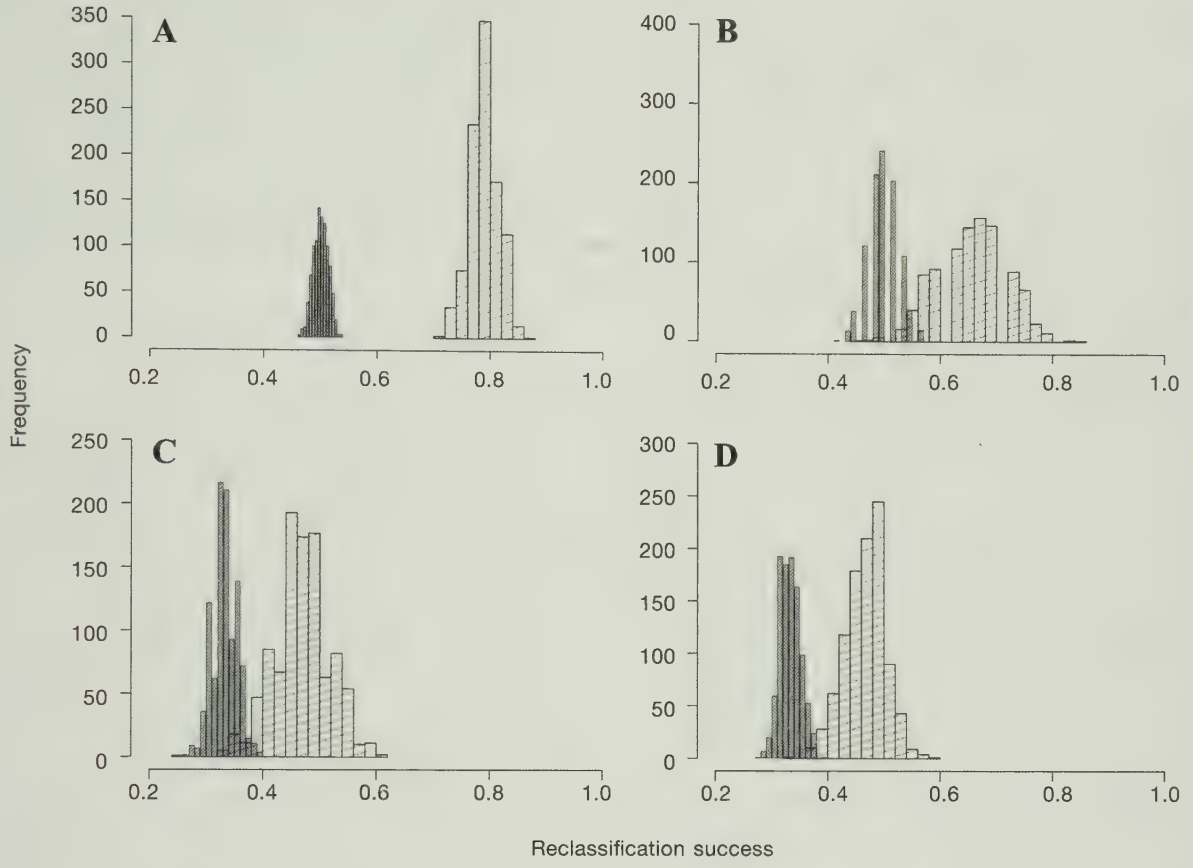

Figure 4

Distributions of randomized reclassification (i.e., null) success rates (shown as the gray bars on the left in each panel) and actual linear discriminant randomization (shown as the hatched bars on the right) for each of 4 geographic analyses of otolith shapes from female southern flounder (*Paralichthys lethostigma*) collected in 1996 and in 2009–12 in 2 basins, the South Atlantic (North Carolina, South Carolina, Georgia, and Florida) and the Gulf of Mexico (Florida, Alabama, Mississippi, Louisiana, and Texas). Distributions are shown for 4 geographic groups: (**A**) both ocean basins, (**B**) Gulf of Mexico states, (**C**) South Atlantic states, and (**D**) North Carolina regions. Only the basin-level analysis indicated clear separation of distributions; each of the within-basin analyses and the within–North Carolina analyses revealed some degree of distributional overlap (illustrated by the gray, hatched bars).

lihood of differing environmental conditions between the U.S. South Atlantic and the Gulf of Mexico led us to hypothesize the existence of distinct otolith shapes for southern flounder at the spatial scale of the basin, and we did detect distinct shapes. On the basis of past observations of high site fidelity from tag-return data in estuarine systems (Monaghan[7]; Craig and Rice[8]), we expected to find differences in otolith shape among fish from separate areas within each basin and even potentially among fish from different estuarine systems within North Carolina. We considered North Carolina a good model with which to test for structure at a finer spatial scale because the inshore waters of the state are made up of a range of system types, from large systems in central and northern regions (e.g., Pamlico and Albemarle Sounds) that contain extensive oligohaline reaches to small, river-based estuaries in the southern region.

Given that even relatively fine-scale shifts in habitat have been observed to generate heterogeneity in otolith shape (e.g., Vignon, 2012), we hypothesized that deviation in geography and hydrography among systems within North Carolina might produce enough environmental variability to influence growth rates and, therefore, the shape of otoliths from southern flounder. However, we detected only a weak signal of spatial structuring in otolith shape of southern flounder among locales within each basin and among regions within North Carolina. We did achieve a rate of reclassification success that was higher than expected in each case, an outcome suggestive of localized environmental effects, but our findings also indicate that

levels of mixing within each basin could be sufficient to largely homogenize these effects. However, we cannot rule out the possibility that some fine-scale structure exists at the within-basin spatial scale for southern flounder. Additional investigations to understand the extent of mixing and its impact on spatial variation in vital rates, such as growth, recruitment, and mortality, will be important to fully comprehend harvest effects and to select the appropriate scale for management.

Within both basins, no interjurisdictional management plan for southern flounder exists, and each state manages its fishery separately. In North Carolina, on the basis of a recent stock assessment, southern flounder are considered overfished with overfishing occurring (Takade-Heumacher and Batsavage[6]), but other states (South Carolina, Georgia, and Florida) have yet to conduct comprehensive stock assessments for flounder in their waters. In North Carolina, the southern flounder has long supported a lucrative commercial fishery (NCDMF, http://portal.ncdenr.org/web/mf/commercial-fishing-harvest-statistics), whereas landings with commercial gear have historically been much lower in the other states. At the same time, the recreational fishery has grown considerably throughout the entire basin; landings have more than doubled since the 1980s (National Marine Fisheries Service, http://www.st.nmfs.noaa.gov/recreational-fisheries/index).

Combined with any increase in participation in commercial harvest activities, the steady growth of the recreational fishery for southern flounder may necessitate that other states develop comprehensive fishery management plans in the near future. In that event, the likelihood that interstate cooperation will be required through a federal fishery management plan (e.g., under the auspices of the Atlantic States or Gulf States Marine Fisheries Commissions) may increase.

Our findings indicate that southern flounder may exist as separate but well-mixed stocks within the South Atlantic and Gulf of Mexico basins. Alternatively, if population structure exists in the form of multiple stocks that differ in their response to harvest, failure to recognize that structure can lead to overexploitation and depletion of less productive and resilient stocks. Therefore, identification of stock structure is essential for accurate estimation of vital rates and stock assessments that promote effective fishery management. Currently, this process is hindered by the lack of understanding of the underlying structure within the population of southern flounder in the U.S. South Atlantic.

Conclusions

Previous studies have found genetic differences between southern flounder occupying South Atlantic and Gulf of Mexico waters, and the results from analyses of otolith shape variation presented here provide further evidence of basin-level differentiation. At finer spatial scales, we detected only limited evidence of population structure inferred from variation of otolith shape to support the existence of separate stocks within basins or within specific regions of a basin (among estuaries in North Carolina). Additional investigation of stock structure will be necessary, integrating these findings with results from other approaches, such as genetic analyses, acoustic or archival tagging, and analysis of spatial variation in life history traits, to determine the appropriate spatial scales to promote effective management and conservation of southern flounder stocks.

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Fish and fishes For papers on taxonomy and biodiversity, the plural of *fish* is *fishes,* by convention. In all other instances, the plural is *fish.*

Examples:

The fishes of Puget Sound [biodiversity is indicated];

The number of fish caught that season [no emphasis on biodiversity];

The fish were caught in trawl nets [no emphasis on biodiversity].

The same logic applies to the use of the words *crab* and *crabs, squid* and *squids,* etc.

Sex For the meaning of male and female, use the word *sex*, not *gender.*

Participles As adjectives, participles must modify a specific noun or pronoun and make sense with that noun or pronoun.

Incorrect:

Using the recruitment model, estimates of age-1 recruitment were determined. [Estimates did not use the recruitment model.]

Correct:

Using the recruitment model, we determined age-1 estimates of recruitment. [The participle now

modifies the word *we,* those who were using the model.]

Incorrect:

Based on the collected data, we concluded that the mortality rate for these fish had increased. [*We* were not based on the collected data.]

Correct:

We concluded on the basis of the collected data that the mortality rate for these fish had increased. [Eliminate the participle and replace it with an adverbial phrase.]

Equations and mathematical symbols should be set from a standard mathematical program (MathType) or tool (Equation Editor in MS Word). LaTex is acceptable for more advanced computations. For mathematical symbols in the general text (α, χ^2, π, $\pm$, etc.), use the symbols provided by the MS Word program and italicize all variables. Do not use photo mode when creating these symbols in the general text.

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